



# ophthalmologica

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7

## VARIATIONS OF LENS THICKNESS IN RELATION TO BIOMICROSCOPIC TYPES OF HUMAN SENILE CATARACT

BY

A. BRUUN LAURSEN and H. FLEDELIUS

The results are reported of 33 ultrasonographic *in vivo* mensurations of intraindividual differences in axial thickness between a cataractous lens in one eye and a biomicroscopically clear or slightly cataractous lens (incipient deep cortical opacity) in the other. Obviously intumescent cataractous lenses were excluded.

In general the cataractous lens was *thinner* than the contralateral clear or slightly cataractous lens.

Large decreases in lens thickness appeared in lenses with the *capsule near opacities* of posterior subcapsular cataract (PSC) + anterior capsular/subcapsular opacity (ACSCO). PSC was more closely correlated to lens thinning than was ACSCO.

Nuclear cataract very often occurred in thin lenses but did not appear to cause lens thinning *per se*.

Deep cortical opacity was not associated with lens thinning.

The present results contributed to our argumentation that the decrease in lens thickness is due to a leak of lens material through the lens membrane beside a possible cessation of growth of the lens fibres.

**Key words:** cataract unilateral human senile - biomicroscopy - ultrasonography - lens thickness intraindividual side differences in - opacities capsule near

It is the aim of the present study to evaluate possible associations between biomicroscopical types of opacities in human senile cataractous lenses and ultrasonographically recorded variations in lens thickness (LT). In particular

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we were interested in clarifying whether anterior capsular/subcapsular opacity (ACSCO) – Bruun Laursen 1976 see Methods below – might be associated with LT changes since ACSCO seems to be a *biomicroscopical* indicator of important *biochemical* changes during senile cataractogenesis in man. Thus ACSCO in human senile cataract has been found to be associated with a) low ribonucleotide pools (Bruun Laursen 1976) b) an increase in lens sodium ion concentration and c) a fall in lens potassium ion concentration (Klauber & Bruun Laursen 1977)

The obvious *macroscopical* characteristic of the cataractous lens is of course its opacification but in addition many cataractous lenses are *smaller* (less voluminous) than clear lenses of coeval persons, as was first shown by Smith (1883). More recently this has been confirmed through investigations into lens wet weights (Maraini & Mangini 1973) and by *in vivo* ultrasonic lens thickness measurements (Babel et al 1969 Delmarcelle & Luyckx-Bacus 1971). The latter authors further included optical pachymetry of the depth of the anterior chamber and roughly examined its possible relations to biomicroscopical types of cataract. They found that the thinnest lenses and the deepest chambers occurred when the lenses were totally opaque.

To our knowledge there have been no reports on the topic dealt with in the present paper. The possible associations between echographically recorded variations in  $\Delta$ LT (the intraindividual side difference in lens thickness in persons with unilateral cataract) and specified cataract morphology

## Material

33 consecutive patients aged 51–83 years with predominantly unilateral senile cataract were selected for the study. Excluded were patients with previous eye injuries or additional eye disease, diabetes mellitus as well as patients receiving corticosteroid treatment. Excluded were also obvious cases of intumescent (Morgagnian) cataract. These exclusions imply that our statements will be valid for non-intumescent cataracts only and not for the full spectrum of human senile cataracts.

We intended to include only strictly unilateral cases of senile cataract but such a material is difficult to collect considering the above criteria of exclusion as well as the age of the patients. Even in a fellow eye with a visual acuity of 1.0 careful examination will as a rule reveal lens opacities in the age group under study. There were however 24 cases of *strictly unilateral* senile cataract. The remaining 11 cases presented marked side differences concerning cataract morphology. In particular we demanded that FSC and ACSCO be absent in the less affected lens. In general this was affected by incipient deep cortical opacity only.

The axial lengths of the eye were used to exclude a possible source of error in the evaluation of  $\Delta$ LT namely a pre-existing anisometropia, now concealed behind

opaque media. In fact, the axial lengths within the 38 pairs of eyes were almost identical the median *side difference* being only 0.2 mm and in all but 3 cases  $\leq 0.4$  mm

## Methods

Three classification systems were used (Bruun Laursen) based on slit lamp examination and ophthalmoscopy according to a) *Site(s) of opacity* within the lens b) *Extent of PSC (posterior subcapsular cataract) and ACSCO* c) An overall impression of the transparency of the lens

### a) Sites of opacity

1) Anterior capsular/subcapsular opacity (ACSCO) whitish dots or an irregular greyish coating apparently located in or immediately beneath the anterior lens capsule (Bruun Laursen 1966)

2) Posterior subcapsular cataract (PSC) posterior subcapsular tufaceous opacity

3) Nuclear cataract grey or brown opacity of the biomicroscopic lens nucleus causing blurred insight to the posterior lens capsule and appearing as a disciform opacity on transillumination with the ophthalmoscope (mydriasis)

4) Cortical cataract irregular or spoke like anterior or posterior *deep* cortical opacities apparently free of the lens capsule

5) Totally opaque lenses uniformly grey or brownish lenses always with extensive ACSCO

b) *Extent of PSC and ACSCO* estimated as percentages of the visible parts of the anterior and posterior lens surfaces respectively

c) *Lens transparency* as estimated roughly on slit lamp and ophthalmoscopic examinations was recorded as

Grade 0 quite clear

Grade 1 a high degree of transparency and a comparatively slight extension of the opacity

Grade 2 more severely affected immature cataract

Grade 3 total lens opacity

The above evaluations were just as the following ultrasound mensurations performed in full mydriasis after instillation of phenylephrine 10% and cyclopentolate 0.5% or tropicamide 1%

*Axial ultrasound measurements* were performed with A mode technique as described by Flodellius (1976) and they were all carried out by him Kretztechnik 1000 a 10 Mc ultrasonolux transducer and a Methocel filled contact glass were used

For the conversion from arbitrary apparatus units to lens thickness in mm we used the intralenticular ultrasound velocity of 1641 m/sec given by Jansson & Kock (1962) for *clear* lenses. The velocity in cataractous lenses has been found to be of the same order 1609 m/sec (Coleman et al 1975). The aberration being less than 1 per cent we did not introduce this latter value in calculations for the cataractous lenses

A pilot study was performed by Flodellius (quoted by Flodellius & Bruun Laursen 1978) 1) to control the US method and 2) to estimate the level of intraindividual



LT differences (right left) in a sample with healthy eyes  $\Delta$ LT values were - as expected - low with a median value  $\leq 0.1$  mm

Statistical comparisons between two types of lens opacity were carried out by means of the Mann Whitney test and correlation analyses by means of the Spearman rank correlation analysis

Table I

Intraindividual side differences in lens thickness (JLT) between a cataractous and a clear lens in 24 persons arranged according to various biomicroscopic types. The individual opacities were compiled regardless of additional opacities in the same lenses. In a few cases the posterior cortex and the posterior capsule of immature cataractous lenses could not be evaluated. *Abbreviations:* PSC posterior subcapsular cataract ACSCO anterior capsular/subcapsular opacity LT = lens thickness. See Methods

Type of opacity	Median $\Delta$ LT mm	Range $\Delta$ LT mm	LT of cataractous lenses		Median age years	N
			Median mm	Range mm		
+PSC+ACSCO	-1.0	-0.4--1.6	4.0	3.6-4.1	69	5
+PSC	-0.5	-0.2--1.6	4.1	3.6-4.5	64	8
+ACSCO	-0.4	-0.1--1.6	4.0	3.6-4.8	69	
-ACSCO	-0.25	0--0.6	4.5	3.9-5.1	64.5	10
-PSC	-0.1	0--0.6	4.6	3.9-5.1	65	8
-PSC-ACSCO	-0.1	0--0.6	4.5	3.9-5.1	66	
Nuclear opacity	-0.4	-0.1--1.6	4.0	3.6-4.9	66	8
-Nuclear opacity	-0.3	0--0.6	4.4	3.9-5.1	64	9
Deep cortical opacity	-0.3	0--1.6	4.5	3.6-5.1	66	17
-Deep cortical opacity	-0.4	-0.5--1.0	4.3	4.0-4.5	60	5
Lens transparency						
Grade 1	0.2	0--0.6	4.5	3.9-5.1	65	11
Grade 2	0	-0.3--1.6	4.0	3.6-4.8	62.5	6
Grade 3 (total opacity)	-0.1	-0.4--1.4	3.9	3.6-4.6	65	

# Results

In the following the results will be viewed from two aspects

First the intraindividual side differences in axial lens thickness ( $\Delta$ LT) will be given in relation to different types of lens opacity. This is done a) for the strictly unilateral cases ( $N = 24$ ) Table I Fig 1) and b) for the combined group of unilateral + bilateral cases ( $N = 38$ ) Table II Figs 2 and 3)

Besides the frequencies of the different kinds of lens opacity in immature cataractous lenses are shown in relation to intraindividual side differences in lens thickness (Tables III and IV) the material being roughly divided into a group of numerically high and a group of numerically low  $\Delta$ LT values

Table II

Differences in intraindividual lens thickness ( $\Delta$ LT) between a cataractous lens on one side and a clear or slightly cataractous lens (with only incipient deep cortical opacity) on the other side again arranged according to biomicroscopic types  $N = 38$

See legend of Table I also (1 lens was inadvertently not graded)

Type of opacity	Median $\Delta$ LT mm	Range $\Delta$ LT mm	LT of cataractous lenses		Median age years	N
			Median mm	Range mm		
PSC+ACSCO	-0.75	-0.4--1.6	3.9	3.4-4.6	61	10
-PSC	-0.6	-0.2--1.6	4.0	3.4-4.6	61	12
ACSCO	-0.65	-0.1--1.6	4.0	3.4-4.8	70	12
-ACSCO	-0.75	0--1.1	4.4	3.5-5.1	65.5	12
-PSC	-0.1	0--0.6	4.5	3.9-5.1	69	9
-PSC-ACSCO	-0.15	0--0.6	4.5	3.9-5.1	67.5	8
Nuclear opacity	-0.6	-0.1--1.6	4.0	3.4-4.8	70	13
-Nuclear opacity	-0.75	0--0.6	4.4	3.9-5.1	65	10
Lens transparency						
Grade 1	-0.7	0--0.6	4.5	3.9-5.1	65.5	12
Grade 2	-0.8	-0.3--1.6	4.0	3.4-4.8	72	11
Grade 3 (total opacity)	-0.6	+0.1--1.4	4.0	3.9-4.6	69	14

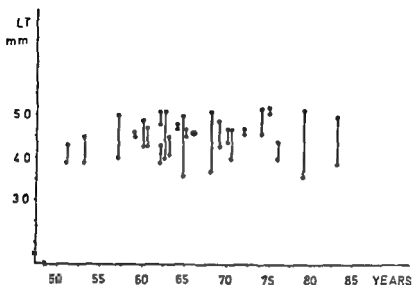


Fig 1

Ultrasonographically recorded intraindividual side differences in lens thickness (ILT) between a cataractous and a clear lens ( $N=24$ ) in relation to age. No significant correlation was found between age and the thickness of the clear lenses in the present age interval of 51-83 years (Spearman's rank correlation coefficient corrected for ties = 0.3%  $0.10 > P > 0.05$ ). ○ clear lens ● cataractous lens

The ILT values will be given with signs as the IT value of the cataractous lens minus the IT value of the clear(er) lens. In most cases the sign is a minus, to signify a deficit in the thickness of the cataractous as compared with the clear(er) lens.

The various lens opacities were of course mingled in many combinations in our material and it is therefore difficult to evaluate the association between the separate opacity and the corresponding ILT. However, this is attempted in Tables I-IV in Figs 2 and 3 as well as in our statistical calculations. Thus the ILT value of an immature cataractous lens with PSC (posterior subcapsular cataract), ACSCO (anterior capsular/subcapsular opacity), cortical and nuclear opacities was used 5 times, namely each time each separate opacity was considered regardless of the 3 additional opacities and when the lens transparency was graded.

Fig 1 shows the individual lens thicknesses (LT) of both eyes of the 24 patients with strictly unilateral cataract. A slight increase in LT with age possibly occurred for the clear lenses. However, the significance level was only  $0.10 > P > 0.05$ .

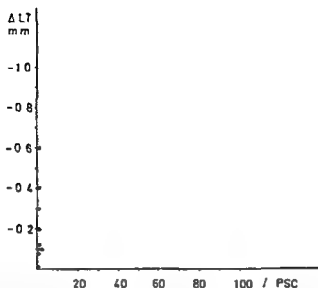


Fig 2

The correlation between the extent of PSC (posterior subcapsular cataract) and  $\Delta LT$  i.e. the intraindividual difference in lens thickness between an immature cataractous lens on one side and a clear or slightly cataractous lens with incipient deep cortical opacity on the other side. Spearman's rank correlation coefficient uncorrected for ties = 0.81  $P < 0.001$   $N = 19$  5 lenses were excluded (compare Table II) because the extent of PSC could not be evaluated

#### Site of opacity degree of opacification

From Table I comprising the group of strictly unilateral cataracts it is obvious that the numerically highest median  $\Delta LT$  values i.e. the thinnest lenses are associated with PSC + ACSCO (-1.0 mm  $P < 0.02$ ) with PSC (-0.5 mm  $P < 0.02$ ) and with grade 2 and 3 cataract (-0.4 mm in both instances  $P < 0.05$  and  $P < 0.01$  respectively). The  $P$  values refer to comparisons with lenses without PSC or ACSCO without PSC and to grade 1 lenses respectively.

The  $\Delta LT$  values of immature cataractous lenses with ACSCO did not differ from those of immature cataractous lenses without this opacity. Nor was there any statistical difference in the strictly unilateral cases as for  $\Delta LT$  values between immature cataractous lenses with and without nuclear opacity or with and without deep cortical opacity.

Isolated deep cortical cataract – the type that occurred in the clearer lens of the almost unilateral cases – presented low  $\Delta LT$  values the median  $\Delta LT$  value for pure cortical grade 1 lenses being -0.1 mm (range = 0 – -0.6

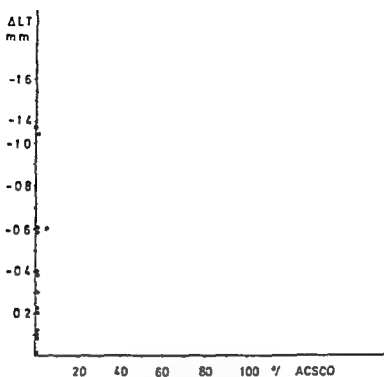


Fig 3

The correlation between the extent of ACSCO (anterior capsular/subcapsular opacity) and IIT (see legend Fig 2) Spearman's rank correlation coefficient uncorrected for ties = 0.59  $0.01 > P > 0.001$   $N = 24$

Table III

Distribution of various biomicroscopic lens opacities in numerically high ( $> 0.6$  mm) and low ( $\leq 0.4$  mm) values of intraindividual side differences in lens thickness (IIT) between an immature cataractous lens and a clear lens in 14 persons. See Legend of Table I also. Median IIT =  $-0.4$  mm

IIT mm	ISC ACSCO	ISC	ACSCO	nuclear opacity	+deep cortical opacity	Grade 1 (grades 1+ to 100%)	N
$\geq 0.6$	60%	80%	60%	60%	60%	40%	5
$\leq 0.4$	18%	36%	33%	42%	5%	15%	10

Table IV

Distribution of various biomicroscopic lens opacities in numerically high ( $\geq 0.6$  mm) and low ( $\leq 0.4$  mm) values of  $\Delta$ LT between an immature cataractous and a clear or slightly cataractous lens with incipient deep cortical cataract  $N=24$ . See legend of Table I also. Median  $\Delta$ LT =  $-0.4$  mm

$\Delta$ LT mm	+PSC+ ACSCO	+PSC	+ACSCO	+Nuclear opacity	Grade 1 (grades 1+2 = 100%)	N
$\geq 0.6$	13%	90%	3%	82%	90%	11
$\leq 0.4$	14%	33%	31%	35%	77%	13

mm not specified in table I) On this background it was considered justifiable to pool the strictly and the almost unilateral cases and the combined results are presented in Table II

The significant differences appearing after statistical evaluation of the material presented in Table I reappear for the findings presented in Table II. Besides the induction in Table II of the 14 almost unilateral cases resulted in additional statistically significant  $\Delta$ LT differences. Thus immature cataractous lenses with PSC + ACSCO ( $-0.75$  mm  $P < 0.01$ ) with PSC ( $-0.6$  mm  $P < 0.01$ ) with ACSCO ( $-0.65$  mm  $P < 0.02$ ) with nuclear opacity ( $-0.6$  mm  $P < 0.02$ ) with grade 2 cataract ( $-0.8$  mm  $P < 0.01$ ) and lenses with grade 3 cataract ( $-0.6$  mm  $P < 0.05$ ) had numerically higher  $\Delta$ LT values - i.e. they were more thinned - than had lens pairs without these opacities. Again for grade 2 and 3 lenses the  $P$  values refer to comparisons with the  $\Delta$ LT values of grade 1 lenses.

In the combined group the  $\Delta$ LT values of immature cataractous lenses with PSC + ACSCO PSC ACSCO nuclear cataract or grade 2 cataract did not differ significantly from the  $\Delta$ LT values of totally opaque (= grade 3) lenses. The above characteristics are also obvious in Tables III and IV which are divided by the size of  $\Delta$ LT. Again lenses with PSC + ACSCO PSC ACSCO nuclear opacity or grade 3 cataract dominate in the numerically high  $\Delta$ LT group (higher degree of lens thinning) and correspondingly they are rare in the numerically low  $\Delta$ LT group. Cortical cataract appears equally often in both groups (Table III).

ACSCO has not been observed as an isolated opacity by the authors neither in this material nor in other patients from the Eye Clinic. In particular it is often combined with PSC: out of 11 immature cataractous lenses with ACSCO in which the posterior capsule could also be estimated 10 had PSC.

As far as the *opacification* of the immature cataractous lenses is concerned nuclear cataract appeared to be more important than did deep cortical opacity. Thus nuclear cataract was found in only 2 out of 12 grade 1 lenses in contrast with 11 out of 11 grade 2 lenses (1 lens was inadvertently not graded). Among the latter 11 lenses a deep cortical cataract was unmistakable in 5 slight only in 2 and absent in 4.

#### **Extent of PSC and ACSCO**

Correlation analysis confirmed the aforementioned results. Positive correlations were found for the *extents* of PSC and ACSCO on the one hand and the numerical size of IIT on the other. This means that the cataractous lenses were more thinned the higher the percentages of PSC and ACSCO were found to be (figs 2 and 3).

### **Discussion**

#### **Associations between degree of lens thinning and biomicroscopic types of cataract**

PSC and ACSCO appeared to be the most important opacities in this context.

Above all a high positive correlation was found between the *extent* of PSC and the decrease in lens thickness. Next ACSCO also showed a definitely positive correlation to the decrease in lens thickness but this may in part at least be due to the common occurrence of PSC in lenses with ACSCO. Thus the IIT differences between strictly unilateral cataracts with and without ACSCO proved to be insignificant.

Lens thinning was more pronounced in grade 2 cataracts (all of which presented nuclear opacity) than in grade 1 cataracts which rarely presented nuclear opacity. This suggests an association of nuclear opacity with lens thinning. However the following observation indicates that nuclear cataract does not cause lens thinning *per se*. The IIT difference between lenses with and without nuclear opacity was insignificant in the strictly unilateral cases.

Keeping constantly in mind the hazards connected with an approach so multifactorial as ours we feel able to state that deep cortical opacity was not associated with the process of lens thinning.

#### **Does a cessation of lens fibre growth or a real decrease in thickness of the cataractous lens account for the side difference in lens thickness?**

Biomicroscopically the smaller lens of cataract has been ascribed to a thinning of the lens cortex (Goldmann & Favre 1961, Brown 1973) while the

thickness of the nucleus of senile cataractous lenses appears to remain constant (Rodriguez Caballero et al 1973). The reason for the thinning of the cataractous lens has been claimed to be a cessation of the (ordinarily continuous) growth of the lens fibres (Goldmann & Niesel 1964) possibly setting in even before lens opacities become visible (Delmarcelle & Luyckx Bacus 1971).

Our study does *not* support the theory of a cessation of lens fibre growth at least not as the only or main reason for the thinning of the cataractous lens. Clear lenses thicken 0.23 mm per decade in the age interval of 20–60 years (Weekers et al 1973) and probably at a somewhat slower rate after the age of 60 years. Provided that the theoretic cessation of lens fibre growth was the only reason for lens thinning some of our cataractous lenses should have stopped growing – in one eye only – already in early adolescence or childhood. Or for our extreme JLT values even *before birth*!

Furthermore the so called normal *fellow eye* in cases of unilateral pre senile/senile cataract frequently develops a similar cataract (with PSC and/or ACSCO) in the course of a few years. In other words the cataract of the first affected eye often remains unilateral for a limited period of time only. In such cases an intraindividual side difference in lens thickness – JLT – should in fact be minimal or absent if the cessation of growth theory were the only valid one. The high JLT values of the present material indicate therefore that the thinning of the cataractous lens cannot be accounted for by a precocious arrest of lens fibre growth only.

All things considered our carefully selected material of largely unilateral cataracts – the fellow eye serving as a control – strongly suggests a *real decrease in lens thickness* in some biomicroscopical types of human senile cataract. In our opinion this is the most fruitful working hypothesis for further research.

**Is the decrease in lens thickness caused by a leak of lens substance into the aqueous?**

The most likely account for a decrease in lens size is an escape of lens material through a lens membrane. The membranes of the lens hypothetically comprise the capsule + the epithelium anteriorly and the lens capsule posteriorly. In this context we would like to draw attention to Friedenwald's (1930) studies. This author found evidence of an increase in permeability of the lens capsule after prolonged exposure of it to cataractous lens cortex. Later it has been suggested that proteins of low molecular weight leak from the lens to the aqueous during senile cataractogenesis (Mach 1963, Charlton & van Heyningen 1968, van Heyningen 1972). In accordance with this Klauber & Bruun



Laursen (1977) found low dry weight (= protein) percentages in totally opaque lenses. Since such lenses are small as compared with e.g. the thicker immature cataractous lenses, totally opaque lenses obviously contain less protein than do thick immature cataractous lenses.

We find it probable that the capsule near opacities of ACSCO and in particular PSC (only a single barrier posteriorly, the lens capsule) may be associated with an increase in lens membrane permeability. To our knowledge, however, no studies have been carried out so far on this topic.

### Concluding Remarks

In summary, our concepts on the decrease in thickness of the biomicroscopically prevailing types of human senile cataract (excluding intumescent lenses) are as follows:

1) In most cases the cataractous lens is definitely *thinner* than the clear lens of the fellow eye.

2) The intraindividual side difference in lens thickness (ILT) is associated with PSC (in particular) and ACSCO and – to a minor extent – with nuclear opacity. Deep cortical cataract appears not to be associated with lens thinning.

3) The ILT is probably accounted for mainly by a real decrease in lens thickness and *not only* by a cessation of lens fibre growth.

4) The decrease in lens thickness may be accounted for by a leak of lens material through the lens membranes.

5) This leak of lens substance appears to be associated with the occurrence of the *capsule near opacities* (PSC and ACSCO).

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## ANT EGG CATARACT

### **A Study of a Family with Dominantly Inherited Congenital (ant egg) Cataract Including a Histological Examination of the Formed Elements**

BY

**STEEN HOLST NISSEN and HENRIK DAA SCHRODER**

A family with ant egg cataract in three generations is described. The cataract is congenital probably of autosomal dominant inheritance. Light microscopy of the ant eggs showed that they are made up of a peripheral zone of lens material and a large almost homogenous centre. Element analysis by  $\lambda$  ray spectrophotometry showed a high content of calcium and phosphorus in the centre. The cataract has been easy to operate on and the postoperative visual results have been good.

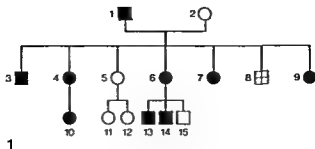
**Key words:** ant egg cataract - zonular cataract - element analysis -  $\lambda$  ray spectralanalysis - heredity

Ant egg cataract is a zonular cataract made up of ant egg like ovoid bodies arranged in a diffuse greyish opaque zone. The ant egg cataract has been previously described by Axenfeld (1900), Stock (1902), Jaeger (1964) and by Riise who in 1967 reported several cases in two generations of one family. Since then we have examined and treated several members of the third generation of this family. The present publication extends the family tree thus contributing to our knowledge about the heredity and at the same time includes a histological study of the ant egg.

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## Ant Egg Cataract



*Fig 1*

Pedigree Black figures patients with ant egg cataract Open figures family members without ant egg cataract Case 8 not examined

## Observations

The cases 1-9 (Fig 1) were described by Ruise (1967) On examination of the heredity we found as illustrated by the pedigree in three consecutive generations that the disease was represented in each generation and in nine out of 15 members Four of these are men One member (case 8) was not examined (died at the age of 15 months) Thus the heredity is seen to be autosomal dominant

## Case Histories

**Case 10 (150471)** A 4 year old girl with failing vision for one year Visual acuity right eye 0.05 left eye 0.1 Dense ant egg cataract was seen in both lenses After aspiration of the cataract in the right eye, and three months later in the left visual acuity was 0.67 in both eyes with correction for aphakia on examination by the patient's own eye specialist

**Case 11 (12091)** A 6 year old girl with visual acuity 1.0 in both eyes without correction Clear lenses Normal ophthalmoscopy

**Case 12 (210913)** A 4 year old girl with visual acuity 0.6 in both eyes without correction Clear lenses Normal ophthalmoscopy

**Case 13 (110171)** A 3 year old boy with reduced vision At the age of three years visual acuity was 0.95 (E chart) in both eyes Examination showed typical ant egg cataract in both lenses After aspiration of the cataract in the right eye and eight months later in the left eye visual acuity was 0.6 in the right eye and 0.5 in the left eye with correction for aphakia Slit lamp examination showed in both eyes scattered ant eggs on the iris In the left eye a fairly dense secondary cataract was seen Normal ophthalmoscopy



Fig. 2  
An eye chart, Case 14

**Case 14 (1973)** A 4-year-old boy with bilateral cataract and an intermittent convergent squint. Sep. 30 visual acuity 0.25 T-chart in both eyes. An incision of the cataract in the right eye was performed and three months later in the left (Fig. 2). Jan. 1975 visual acuity was 0.6 in the right eye and 0.3 in the left with correction for aphakia. Slit lamp examination showed secondary cataract and a couple of white eggs on the iris. The extracted material from the left lens was studied by light microscopy and electron analysis.

**Case 15 (1974)** Examination difficult due to lack of cooperation. No complaints or signs of reduced vision. Clear lenses and a normal fundus were observed on examination with an ortho-lamp.

## Method

The extracted material from the cataract was fixed in cold 3% glutaraldehyde with 0.1 M phosphate buffer (pH 7.2). After three h fixation the tissue was post-fixed for one h in 1%  $\text{OsO}_4$  followed by dehydration in graded alcohol and embedded in Epon 812. Crude zinnver as well as dissected anti-eggs were embedded. Some tissue was decalcified for five days in 10% EDTA in 0.1 M tris buffer (pH 7.2) before postfixation and embedding.

For light microscopy 2  $\mu\text{m}$  thick sections were stained with toluidine blue.

Some Epon blocks with an egg in the cut surface were coated with silver for porosity technique and analyzed for elemental content in a JEOL JAX-50A electron microprobe equipped with an Ortec energy dispersive X-ray spectrometer.

**Light microscopy** The characteristic finding was that of globular structures with a diameter of about 500  $\mu\text{m}$  (Figs. 3 and 4). They consisted of a major central greenish area which was stained weakly by toluidine blue and  $\text{OsO}_4$ . Much of the core disintegrated during sectioning.

Surrounding the core lightly stained homogenous lens material was seen

At the border between the core and the surrounding area a zone containing densely stained material often organised in waved formed strands was seen (Fig 3) The border zone interdigitated with both the core and the surroundings In decalcified material the central core was preserved and was seen to consist of a heavily stainable material (Fig 4)

*Analysis for elements* The ant egg appeared as a bright area in the backscattered electron image (BEI) (Fig 5) Energy dispersive analyses from different points of the ant egg showed a content of calcium and phosphorus X ray scan for calcium and phosphorus across the structure demonstrated that the localization of these elements coincided with the bright area of the BEI (Fig 5)



*Figs 3 and 4*

*Fig 3* Isolated ant egg Most of the calcified central zone is lost Rests of the unstainable material are seen at the open arrow At the thick arrow the wavy structure of the border zone is seen 3  $\mu$ m toluidine blue  $\times 380$

*Fig 4*

*Fig 4* Isolated decalcified ant eggs The organic material of the central zone can here be demonstrated The wavy border zone is seen at the thick arrow 3  $\mu$ m toluidine blue  $\times 380$



*Fig. 5*

Backscattered electron image (BFI) of ant egg (ae) with superimposed plot of Ca and P concentrations obtained with line scanning. BFI shows the size and shape of the ant-egg. The scanning is performed along the line SL. Base line is seen at the bottom of the picture  $\times 350$ .

### Discussion

Ant egg cataract is a rare form of congenital cataract characterised by ovoid whitish structures arranged in a zonular area, often most frequent in the anterior part of the lens (Fig. 2). After surgical aspiration of the lens material, the individual ant eggs were examined by light microscopy and by energy dispersive element analysis. Electronmicroscopic observations of the ant eggs will be published in a subsequent paper.

Ant egg cataract manifests itself clinically by considerable reduction of sight in early childhood. The patients described in this article were operated on at the age of 3-4 years. There have been no technical difficulties attached to the operation: the ant eggs can be aspirated at the time of operation without any difficulty. As long as there is no secondary cataract, the vision was found to be good.

On cutting ant eggs with a glass knife for semi-thin sections, the knife broke several times. Thus the material was extremely hard.

## *Ant Egg Cataract*

Stock (1902) studied by chemical and histochemical methods some crystal pearls of lens origin corresponding to the present ant eggs. He observed that the pearls were insoluble in alkaline solutions in alcohol ether and chloroform but he found development of gas and dissolution of some components of the pearls during treatment with hydrochloric acid. These results pointed to a content of calcium carbonate in the crystal pearls. The histochemical staining methods for calcium phosphate (v. Kossa) and silver nitrate employed by Stock did not demonstrate the presence of this compound.

The multi elements analysis employed in the present study does not analyse for carbon; moreover a scan for carbon would not distinguish between organic and inorganic carbon; thus carbonate may be present in the ant egg. However, contrary to Stock's observation, a content of phosphorus together with calcium was found by the energy dispersive analysis.

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## **HUMAN TRAUMATIC CATARACT A Quantitative Microradiographic and Electron Microscopic Study**

BY

**P P FAGERHOLM and B T PHILIPSON**

Six cases representing different stages of cataract formation secondary to eye trauma were subjected to quantitative microradiographic or electron microscopic examination. Anterior and posterior subcapsular cataracts were found to contain extensively swollen lens fibers in the subcapsular cortex. Microradiographic measurements revealed a reduced concentration of dry mass in the subcapsular cortex around the whole circumference of the lens. The inner cortex and the nucleus appeared normal both microradiographically and electron microscopically.

Two of the examined cases had an opaque cataract membrane and one had a Soemmerring's ring. The opaque membranes consisted of irregular mass of degenerated lens fiber material as well as regenerated lens epithelial cells. A wide range of dry mass concentration was found in the opaque membrane that was studied microradiographically. Alterations in morphology and dry mass concentration are more than sufficient to explain the development of opacification in traumatic cataract.

**Key words:** human lens - traumatic cataract - after cataract - electron microscopy - quantitative microradiography - light scattering

Trauma to the lens can lead to the formation of cataract. Post trauma pathology may range from a local opacity at the site of the lens injury to an almost total resorption of the lens leaving nothing but a membranous after cataract (Davidson 1940a, b; Duke Elder 1912).

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The end result of the post traumatic events is dependent on various factors such as the age of the patient and the site and size of the injury. In general a minute injury will result in only a local opacification (Keeney 1971). A larger wound will cause formation of a posterior subcapsular cataract which often leads to a swollen and totally opaque lens. If the cataract is not aspirated at this stage the major part of the lens fiber mass will be resorbed.

In an old lens the hard nucleus is generally resistant to a complete breakdown. The anterior lens capsule with its epithelium and the posterior capsule remain intact and may form a capsular bag enclosing cortical material and regenerated epithelium. Furthermore regenerating lens epithelium can form an after cataract similar to that seen following extracapsular cataract surgery. The ultrastructure of two human traumatic cataracts secondary to penetrating injuries has earlier been studied by Rafferty et al (1974).

In this study different stages of cataract formation secondary to eye trauma were subjected to quantitative microradiographic or electron microscopic examination. The observations are compared to recent results obtained in experimentally induced traumatic cataract (Fagerholm & Philipson 1978a, b).

## Materials and Methods

The lens specimens studied were selected in order to illustrate different stages of traumatic cataract. Within each stage there was a random selection of the cases analysed. (For detailed information see case reports). All material was obtained from the Department of Ophthalmology, Karolinska Hospital, Stockholm in connection with eye surgery performed to restore vision.

The lens material was processed within 30 min following extraction after having been transported in Eagles MEM tissue culture medium.

All lens material was examined in a Haag Streit 800 slit lamp microscope (6–40 $\times$ ) prior to extraction. Before processing the tissue further examination was performed in a Wild 8M stereomicroscope.

### Quantitative microradiography

The lens specimens were frozen in isopentane precooled in liquid nitrogen to  $-140^{\circ}\text{C}$ , freeze sectioned into 10–20  $\mu\text{m}$  thick sections and freeze dried. Together with a reference system the sections were mounted in close contact with a fine grained photographic emulsion and exposed to soft X rays generated at 3 kV. The microradiograms thus obtained were evaluated densitometrically in order to determine the dry weight of different regions of the lens. (For detailed information about the technique see Philipson 1969).

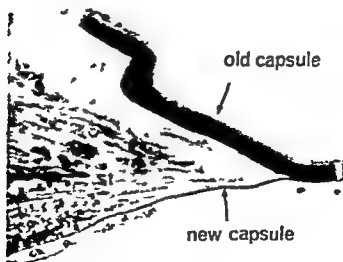
### Light and electron microscopy

The lens material was fixed in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 30 min. After postfixation in 1% osmium tetroxide and dehydration the specimen was embedded in Epon via propyleneoxide. Ultrathin sectioning was performed on a LKB microtome. The sections were stained in 3% uranylacetate and 5% lead citrate and examined in a Philips 301 electron microscope. 1  $\mu$ m thick sections were used in light microscopy examination.

## Case Reports and Observations

### Case 1

A 13 year old boy had at the age of 6 years suffered from perforation of the right eye by a thin needle. A local epiphora was seen at the site of the wound soon after trauma. A dense posterior subcapsular cataract then developed reducing visual acuity to less than 0.1. Cataract aspiration was performed after an anterior capsulotomy.



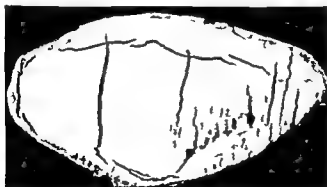
*Fig. 1*

Light micrograph from a wound area obtained from a lens that seven years earlier suffered from a perforation injury (Case 1). Epithelial cells have regenerated and formed a new thin capsule. Outside the regenerated epithelium a scar tissue is seen. Toluidine blue.  $\times 300$ .

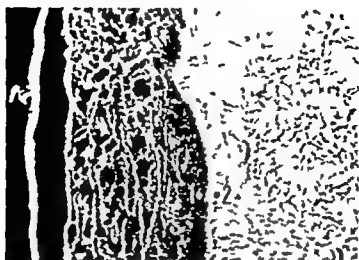


Electron micrograph from the central part of the scar tissue (Case 1) Around elongated cells layers of fibrous material is seen forming a large extracellular space  $\times 2200$

Light and electron microscopy of the excised part of the anterior capsule revealed a healed lens wound. Scar tissue was seen between the rolled up capsular flaps (Fig 1). This tissue consisted mainly of elongated cells surrounded by abundant extracellular material (Fig 2). Underneath this scar tissue a single layer of regenerated epithelial cells was seen. These cells had formed a new lens capsule.



Microradiograph from a central section of a lens with traumatic cataract (Case 2). The lens had a thin zone of opacification in the subcapsular cortex. A thin zone with low X-ray absorption about 0.1 mm wide is seen in the entire subcapsular area  $\times 100$



*Fig. 4*

Microradiograph from the same lens as in Fig. 3. Detail from the posterior subcapsular cortex reveals a reduced dry mass content in the outer zone compared to the inner zone. The formation of swollen cells is obvious. Dry mass determinations are given as a mean value and a 99% confidence interval of 10 measurements at each point.

$$1.007 \pm 0.03 \text{ g cm}^{-3} \quad 2.035 \pm 0.05 \text{ g cm}^{-3} \times 2.5$$

## Case 2

This 54 year old man's left eye had been perforated 22 years earlier by the edge of a steel band which caused a minor lens injury. A slight posterior capsular opacification developed during the first year reducing visual acuity to 0.9. A later development of central retinal detachment reduced visual acuity to light perception. Finally a therapy resistant glaucoma developed and the eye was enucleated. In spite of these complications the lens appearance had not changed, the slight posterior opacification not having progressed any further.

Densitometrical evaluation of microradiograms obtained from lens sections of the region close to the optic axis revealed a low concentration of dry mass in the subcapsular cortex (Fig. 3). This was found around the whole circumference of the lens. Fig. 4 shows the results of the densitometrical evaluation. A very steep gradient in the concentration of dry mass was present at the borderline between this subcapsular zone and the inner cortex. The inner cortex and the nucleus had normal concentrations of dry mass. The subcapsular zone containing swollen lens fibers varied in thickness between 0.05 and 0.15 mm.

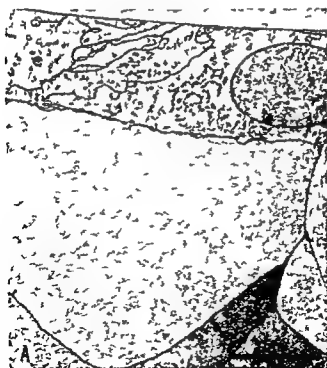
**Case 3**

A 33 year old man had been hit in his left eye by the handle of a pair of garden scissors six months prior to cataract extraction. This blunt trauma caused backward lens subluxation and a herniation of the vitreous. A posterior subcapsular cataract developed the first months and remained relatively stationary until the time of surgery. Visual acuity was reduced to 0.1.

Extensively swollen lens fibers were most conspicuous in the anterior and posterior subcapsular cortex (Fig. 5). The lens epithelium appeared normal except in one region near the equator where a multilayered arrangement of the cells were seen (Fig. 6). No typical scar tissue could be found.

**Case 4**

A 30 year old man had 19 years earlier suffered from a perforation of the left eye caused by a thorn. A progressive traumatic cataract developed. At the time of surgery



*Fig. 5*

Electron micrograph from a human lens with traumatic cataract (Case 3). The epithelium (e) appears normal but lens fibers underneath are enlarged  $\times 4200$ .



Fig 6

Light micrograph near the equator from the same lens as in Fig 5 (Case 3). A multilayered epithelium is seen. Lens fibers are slightly swollen underneath. Toluidine blue.  $\times 160$

most lens material had been resorbed and the remaining tissue formed a dense opaque membrane reducing visual acuity to perception and localization of light. The central part of this opaque after cataract was surgically extracted to restore vision.

The biomicroscopic appearance of the after cataract and the electron microscopic findings were very similar to those of case 5. They will therefore be presented together with the observations made in case 5.

#### Case 5

A 39 year old man had 70 years earlier suffered from a perforating nail injury to the right eye. The nail had injured the lens in its anterior pole. A traumatic cataract developed immediately. The lens material eventually resorbed leaving only an after cataract consisting of a dense opaque membrane. Visual acuity was reduced to perception and localization of light. The central part of the membrane was surgically excised to restore vision.

The opaque membranes excised in cases 4 and 5 had a similar appearance in the electron microscope. The material was found to consist of two main components:

- a) degenerated lens fiber material (Fig 7) and
- b) regenerated lens epithelial cells surrounded by abundant extracellular material (Fig 8). The degenerated fibers consisted of membrane enclosed spheres containing a substance of low stainability. Membrane degradation products with myelin like appearance were seen. Membrane remnants from adjacent

cells were also observed. In the parts where regeneration had taken place the tissue consisted of elongated cells at different stages of degeneration surrounded by a large extracellular space. This space was filled with layers of fibrillar material parallel to the cells.

#### Case 6

A 74 year old woman was killed in a car accident. The eyes were obtained about 4 h post mortem for the purpose of corneal grafting. On examination one eye was discarded because of a corneal scar and an anterior synchia. It was later established that the donor had suffered from a penetrating knife injury to the eye about 10 years earlier. In place of the lens an after cataract was found consisting of a ring shaped semi transparent structure (a Soemmerring's ring) connected to a central opaque membrane.

The microradiographic appearance of this after cataract was examined (Fig. 9). The ring shaped structure was covered by a capsule. The material enclosed by the capsule consisted of lens epithelium and relatively intact lens cells. Quantitative measurements of the concentration of dry mass revealed an even increase from the peripheral parts to the center of the Soemmerring's ring. The highest values  $0.44 \text{ g cm}^{-3}$  were found in the central region of the ring (Fig. 9). The opaque membrane within the Soemmerring's ring consisted of irregular masses of lens material with a wide range of dry mass concentration (Fig. 9).

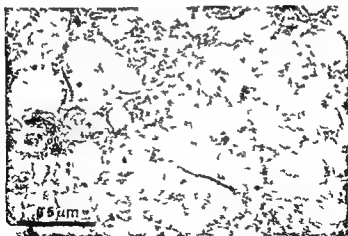


Fig. 1

Electron micrograph from a central part of an after cataract membrane (Case 5). Only scarce membranes are seen sometimes surrounding globuli with an amorphous less electron dense material. The membranes are often discontinuous consisting mainly of junctions  $\times 40,000$ .





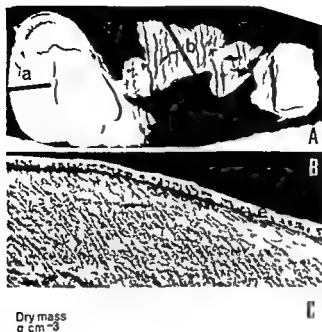


Fig 9

Micrograph from a section through a ring of Soemmerring (A) Densitometrical determinations have been performed along bar a and bar b (shown in C)  $\times 11$  B At the periphery an epithelial like structure (c) is seen inside a thin capsule  $\times 12$  C Diagram of the dry mass content along bar a and b in A The mean value and a 99% confidence interval of 10 determinations at each point is given.

A further progression of the subcapsular cataract will cause a totally opaque lens with a swollen cortex. Finally the lens will rupture. In younger individuals the major part of the lens substance will be resorbed. Generally the posterior lens capsule is left forming an after cataract (Cases 4 and 5). The posterior



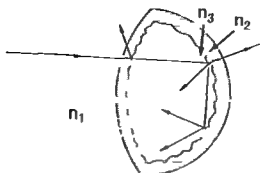


Fig 10

Schematic drawing of a lens with subcapsular reduction of dry mass concentration similar to that shown in Fig 9. Light scattering might occur at the interface between regions with different refractive indices. The refractive indices given are calculated from determinations presented in Fig 4. Aqueous  $n_1 \approx 1.33$ , hydrated cortex  $n_2 \approx 1.35$  and normal cortex  $n_3 \approx 1.40$ . One ray of light is marked and the main sources of light scattering are indicated. The scattering of light is dependent on the angle of incidence. Thus for these refractive indices the critical angle where total reflection occurs is 15 degrees. At this angle total reflection occurs at the posterior interface between normal and hydrated cortex. This posterior interface is wavy and irregular. Thus the incident light frequently hits this interface at high angles causing an intense light scattering.

Soemmerring's ring is often accompanied by a central opaque membrane which is irregular in its morphology and large local variations in dry mass probably intense light scattering. This central membrane probably represents an after cataract membrane similar to those in Cases 4 and 5.

A sharp interface was seen in Case 2 between areas with normal and reduced dry mass. The interface found at the borderlines between transparent and opaque cortex corresponds to a jump in refractive index from about 1.35 to about 1.40. This change in refractive properties is sufficiently large to cause an intensive scattering of light especially if, as in Case 2, the interface is irregular. Light will then frequently hit the interface at angles close to the critical angle and consequently be totally reflected. In Fig 10 a schematic example of this light scattering is shown. Thus the physical background of the opacification with sharp jumps in the concentration of dry mass. In the normal human lens local reductions of dry mass content are never found (Philipson & Fagerholm 1973). The morphological alterations discussed above along with the subsequent local reduction in the dry mass concentration are more than sufficient to explain the development of opacifications in traumatic cataract.



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## LATE COMPLICATIONS OF 7-0 POLYGLYCOLIC (DEXON) SUTURES IN CATARACT SURGERY

BY

ANNELI KLEMETTI

The late complications of corneo scleral wound healing after the 10th postoperative day were recorded in 103 routine adult cataract operations using 7-0 polyglycolic acid (Dexon®) sutures and compared with 80 routine adult cataract operations using 9-0 monofilament nylon (9-10 Ethilon Nylon®) sutures

In 37 eyes of the 7-0 Dexon group filtrating blebs gaping of the corneo scleral wound anterior chamber collapse or decrease in the intraocular pressure were observed compared with one filtrating bleb in the 9-0 nylon group The majority of late complications occurred 92 to 42 days after surgery Ten filtrating blebs remained after a 3 months follow up period The most probable cause of the late complications in corneo scleral wound healing was discussed

*Key words:* cataract surgery late complications - polyglycolic acid sutures Dexon®

The advantages of polyglycolic acid sutures (Dexon®) compared with many other suture materials are evident The good handling characteristics a diameter smaller than that of other absorbable sutures of equivalent tensile strength the non antigenic nature the minimal tissue reaction and the uniform and predictable absorption have made polyglycolic acid sutures useful in both intra and extraocular surgery (Furguele 1974 Sugar et al 1974 Sugar 1975 Williamson 1974 Helveston & Callahan 1976 Merrit et al 1974 White & Parks 1974)

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From experimental studies both after implantation in tissues other than the eye and after extracocular muscle surgery it appears that the tensile strength of polyglycolic acid sutures follows a uniform progressively decreasing pattern (Katz & Turner 1940 Craig et al 1975 Apt et al 1976)

In clinical series less attention has been paid to late complications which may be due to loss of tensile strength of the suture. The purpose of this study was to determine the late complications (15 days or more after surgery) of cataract extractions using 7-0 polyglycolic acid sutures (7-0 Dexon®) compared with the use of 9-0 monofilament nylon sutures (9-0 Ethilon Nylon®), and to pay special attention to insufficiency in the healing wound (gaping and filtrations of the wound anterior chamber collapse and decreased intraocular pressure)

Dexon® is the registered trademark of Davis & Cook American Cyanamid Company Pearl River N Y 10955

Ethilon Nylon® is the registered trademark of Ethicon Inc. Somerville N J USA

### Material and Method

In 1976 all routine adult cataract operations in the Eye Department of the Central Hospital of Middle Finland were performed using 7-0 polyglycolic acid green sutures (7-0 Dexon®) with 35 Circle Lancet Needle 11 2 during the first six months and 9-0 monofilament nylon sutures (9-0 Ethilon Nylon®) with micro point cutting C needle during the remaining six months. Of the total 203 cataracts 13 9-0 Ethilon Nylon and 2 7-0 Dexon cataracts were omitted because of difference in the suturation technique. In the remaining 188 cataract operations 7-0 Dexon sutures were used in 108 eyes and 9-0 Ethilon Nylon sutures in 80 eyes. The operation technique is presented in Table 1. All patients were operated upon under local anaesthesia using 0.5% lidocain chloride with 1:200 000 adrenaline (epinephrine Lidocain Adrenalin®) for retrobulbar injection and to produce facial block. Lidocain Adrenalin® is the registered trademark of Orion Helsinki 51. The operations were performed using Keeler's wide angle operating telescopic spectacles (magnification  $\times 2$  or  $\times 4$ ).

All 188 patients except one in the 9-0 Ethilon Nylon group had limbus based conjunctival flaps dissected followed by limboscleral incision with standard razor blade fragment (Keeler).

The wound was widened with corneal scissors to 160–180°. The number of 7-0 Dexon and 9-0 Ethilon Nylon sutures used in the corneo scleral wound were 6 to 10 placed interrupted.

*Late Complications of 7-0 Dexon Sutures*

*Table 1*

Operation techniques of 103 adult cataracts using 7-0 Dexon sutures and of 80 adult cataract using 9-0 Ethilon Nylon sutures in the closure of the corneo scleral wound

	7-0 Dexon (103 eyes)	9-0 Ethilon Nylon (80 eyes)
Limbus based conjunctival flap	103	79
Corneo scleral incision	103	79
corneal incision		1
Basal iridectomy	51	41
peripheral iridectomy	31	10
meridional iridotomy	26	29
Number of sutures	6	4
(corneo scleral wound)	7	38
	8	19
	9	16
	10	7
Alpha chymotrypsin		
used 0.1-1.0 ml	44	20
not used	64	60
Intracapsular extraction	103	74
Extracapsular extraction (planned)	3	3
Extracapsular extraction (not planned)	-	3
Extraction with cryophobe	103	76
Extraction with irisphake	-	1
Extracapsular technique	5	3
Vitrectomy anterior	5	4
At end of operation		
Anterior chamber deepened with balanced salt solution	80	50
Anterior chamber deep without balanced salt solution	27	21
Anterior chamber flat	1	-
Closure of conjunctival flap continued 6-0 plain catgut	103	49



The depth of placement of the sutures was approximately  $\frac{1}{2}$  of the corneo-scleral tissue. Of the corneo-scleral sutures usually three were preplaced prior to lens extraction.

The 7-0 Dexon sutures were tied with a double loop knot followed by two square knots. The 9-0 Ethilon Nylon sutures were tied with a treble loop knot followed by two square knots. Beginning with the first postoperative day all patients received 0.1% dexamethasone drops four times daily and 1% atropine sulphate twice daily during the hospital stay (usually 6 to 8 days).

The intraocular pressure was measured as a standard procedure on the 2nd, 4th and 6th day after surgery. Acetazolamide was used systemically when the intraocular pressure exceeded 22 mmHg. The first examination after discharge from hospital was performed 15 to 30 days postoperatively and the follow-up examinations thereafter at 1, 2 and 3 to 6 months intervals counting from the operation. The whole material consisted of 66 males and 105 females in the 7-0 Dexon and the 9-0 Ethilon Nylon group together ranging in age from 22 to 92 years. No difference in the ages of the 7-0 Dexon and 9-0 Ethilon Nylon groups could be observed (Table II).

There were seven patients with both eyes operated using 7-0 Dexon sutures and three patients with both eyes operated using 9-0 Ethilon Nylon sutures. Seven patients had one eye operated with 7-0 Dexon sutures and the other eye with 9-0 Ethilon Nylon sutures. The complications observed were verified by slit lamp microscopy. Only those cases in which the complications appeared

*Table II*

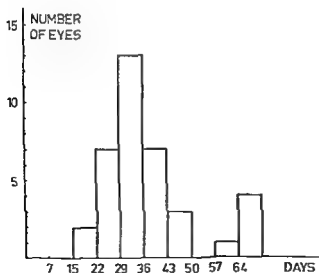
The age distribution of the patients in the 7-0 Dexon and 9-0 Ethilon Nylon groups

Age	7-0 Dexon (105 eyes)	9-0 Ethilon Nylon (80 eyes)
20-29 years	1	
30-39	-	
40-49	5	
50-59	21	4
60-69	33	9
70-79	34	5
80-89	19	14
90-99	1	1

*Table III*

The late complications observed in the 1-0 Dexon group and the 9-10 Ethilon Nylon group

	1-0 Dexon (105 eyes)	9-10 Ethilon Nylon (80 eyes)
Filtrating bleb and hypotony	17	1
Hypotony $\leq 6$ mmHg	9	-
Suspected gaping of the corneo scleral wound and moderate hypotony 1-10 mmHg	8	-
Uveal prolapse	1	-
Choroidal detachment and hypotony	1	-
Intravitreal haemorrhage and hypotony	1	-
	37 (34.3%)	1 (1.3%)



*Fig 1*

The time of appearance of recorded late complications in the 1-0 Dexon group

15 days or more after surgery were included in the group of late complications. None of these eyes showed any sign of wound insufficiency (filtering bleb gaping of the wound, anterior chamber collapse or decrease in intraocular pressure) at the time of discharge from hospital or at examinations before the 15th postoperative day.

## Results

Table III summarizes the late complications observed after the 15th postoperative day in both the 7-0 Dexon and the 9-0 Ethilon Nylon group. In the 7-0 Dexon series 3% eyes with late complications were registered and in the 9-0 Ethilon Nylon series complications were seen in only one eye. The time of appearance of recorded complications is summarized in Fig. 1. The earliest complications in the 7-0 Dexon group (2 cases) were detected 21 days postoperatively. The majority of complications relating to the wound were however recorded between the 22nd and 42nd days after cataract extraction (3 eyes). The only filtering bleb in the 9-0 Ethilon Nylon group was not recorded until the follow up examination nine months after operation. At the examinations one and two months postoperatively no filtration was observed.

The follow up of the whole material is presented in Table IV.

Follow up became more difficult because of the advanced age and poor health of many of the patients. Seven patients died during the follow up period.

*Table II*  
The follow up period of the whole material

Months	7-0 Dexon whole series (104 eyes)	9-0 Ethilon Nylon whole series (80 eyes)
1-2	14	11
3-4	20	5
5-6	21	13
7-8	24	12
9-10	9	5
11-12	13	7
13-	8	21

Of the patients in the 7-0 Dexon group 10% have been followed up for five months or more and for the 9-0 Ethilon Nylon group the follow up percentage is 75

Ten of the 17 filtering blebs with 7-0 Dexon cataract included in the group of late complications still had the filtering bleb after a five to 11 months observation period. In four cases the filtering bleb disappeared spontaneously and in three cases the follow up period was less than five months. Reoperation has been performed in one eye of the 7-0 Dexon group (a transconjunctival cryoapplication).

No differences between the 7-0 Dexon and 9-0 Ethilon Nylon groups were observed with regard to the increased intraocular pressure on the 2nd and 6th day after operation, use of alpha chymotrypsin or number of sutures in the corneo-scleral wound. No statistical evaluations of these results were performed because of the small number of cases in the groups studied.

### Discussion

The filtering blebs reported by Sugar (1975) in 11 cases of 156 cataracts with 6-0 Dexon sutures and in four cases of 70 cataracts with 7-0 Dexon sutures followed for six weeks to 1½ years were more frequent in the earlier performed operations. Those cases were attributed to the disproportionately large needle size compared with the suture diameter. Most of the blebs were supposed to be due to variations in the depth of placement and too tight suturing.

In these series the manufacturer's directions concerning the suturation technique were followed exactly and the needle size (3/8 circle Lancet Needle LE 2) was adequately proportioned to the 7-0 Dexon suture.

None of the eyes in which late complications of the 7-0 Dexon group were recorded showed any sign of insufficiency of the wound at the time of discharge from hospital. Also the comparison of the late complications of the 7-0 Dexon group with those of the 9-0 Ethilon Nylon group (37 cases and 1 case respectively) excludes the probability of failure in wound closure.

Very interesting is the time of occurrence of the late complications. The earliest complications were recorded on the 21st postoperative day and in most cases the complications were registered 22 to 42 days after surgery.

Experimentally Katz & Turner (1970) have shown that the *in vivo* tensile strengths of 4-0, 3-0, 2-0, 0 and 1 size of Dexon sutures implanted subcutaneously in rabbits had decreased 50% at 11 days and about 80% at 15 days. The studies with rats by Craig et al (1975) indicate that both 2-0 and 4-0 size Dexon sutures retain the high original straight pull strength at seven

days postoperatively but then undergo a sharp decline in strength. At 28 days after surgery there is no measurable breaking strength left. The tensile strength of 5-0 Dexon sutures after extraocular muscle surgery in rabbits gives a similar result (Apt et al 1976).

The clinical manifestations of corneo scleral wound insufficiency in these series correspond to the experimental observations. The results speak for the presumption that 5-0 Dexon sutures lose their tensile strength too rapidly before healing of the corneo scleral wound is completed. Another possibility could perhaps be the suture technique (too tight suturing). Without histological examination the possibility of necrosis in the suture canal as the primary cause cannot be excluded. However in the light of these results 5-0 Dexon sutures cannot be recommended in cataract operations in which the long term retention of suture tensile strength is essential for wound healing.

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## CHANGES IN PUPILLARY DYNAMICS IN YOUNG MEN DURING PROLONGED SEVERE EXERCISE

BY

RAGNAR LUND KARLSEN and NILS SOLI

Infrared pupillography has been performed on 9 male cadets taking part in a combat course lasting for 5 days. The combat course involved lack of sleep, strong physical exercise and caloric deficit. During the course pupillary size was determined after rapid adaptation to darkness and the rate of contraction and the rate of dilatation after stimulation of the opposite eye with light was determined. Dilatation time increased by 50% towards the end of the course and pupillary size before light stimulation was reduced by 14%. On day 3 and 5 but not on day 1 small amplitude pupillary oscillations were observed. The speed of contraction was however unchanged.

*Key words:* pupillary dynamics; multifactorial stress

Each year cadets from the Norwegian Academy of War participate in a combat course lasting for 5 days. In a similar course (1976) Opstad et al (1978) found by clinical examination that pupillary dynamics might be affected towards the end of the course. It has in addition been reported (Yoss et al 1970) that lack of sleep is accompanied by small amplitude pupillary oscillations and reduced pupillary size. This study was therefore undertaken to see how the pupils were affected by a severe multifactorial stress.

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## Materials and Methods

The subjects of this investigation were 9 male cadets of the Norwegian Military Academy participating in a ranger training course from Monday 12th (Day 1) June 1978 at 5 a.m. till Friday 16th (Day 5) June 1978 at 6 p.m. The cadets were between 22 and 25 years old (mean  $24.2 \pm 0.4$ ) and had studied at the Academy for about one year. They were healthy and in excellent physical and mental condition. Due to slight injuries during the course two cadets could not be examined on day 3 and one on day 5.

During the course the cadets were exposed to a combined stress of physical strain, sleep deprivation and caloric deficit. Previous work has shown that the physical exercise of the cadets was equivalent to 8–10 000 kcal/day (Waldum & Huser 1974). The amount of sleep was estimated to be 1–2 h totally during the course. This estimate fits well with continuous pulse registrations during previous courses (Waldum & Huser 1974).

The course took place at about 500 m altitude in a forest area in the eastern part of Norway. The weather was mostly good and was sunny and warm during the days (20–25°C) and cool at night (4–8°C). On day 1 and day 5 the cadets were tested at 5 a.m. in the same room (without windows) at a military camp. On day 1 after 7 h of regular sleep. On day 3 (when the course had lasted for 48 h) they were tested under more improvised conditions in a forest cabin at 9 a.m.

**Pupillographic studies.** A Grundig TV camera, model FA 70, was connected to a Sony Video Tape recorder, model AV 3670 CE, and an ordinary Sony TV monitor. This combination made it possible for the examiner to simultaneously watch the eye on the TV screen and record on the video tape. The display of a digital watch was placed temporal to the eye for time determination and was recorded simultaneously. This made it possible to determine pupillary size as a function of time with an accuracy of 0.1 sec.

**Light conditions.** The examination was performed in darkness and the eye was illuminated with infrared light. Due to the military program of the course not more than 10 min was available for each examination, not allowing complete dark adaptation to occur.

**Light stimulation.** The contralateral eye was stimulated with a flash of centered light from a 20 W light bulb placed 0.5 m from the eye. Light stimulation lasted for  $1 \pm 0.2$  sec and was repeated three times with 1 min interval. The switch of the light bulb was connected to the TV camera giving a signal to the tape recorder when the light was on.

*Table 1*  
Effects of combined severe stress on pupillary dynamics studied by infrared pupillography on male cadets

	Pupillary size before light $A_1$ mm <sup>2</sup>	Pupillary size after light $A$ mm	$A_1 - A$ mm <sup>2</sup>	Half time of contraction in sec	Half time of dilatation in sec	Number of cadets
Day 1	$33.4 \pm 0.8$	$15.6 \pm 0.8$	$19.3 \pm 1.7$	$0.61 \pm 0.03$	$1.13 \pm 0.17$	9
Day 3	$36.6 \pm 0.9^*$	$14.9 \pm 0.1^*$	$11.7 \pm 1.6$	$0.57 \pm 0.03$	$1.41 \pm 0.18$	7
Day 5	$38.9 \pm 2.8^*$	$13.0 \pm 1.4$	$15.9 \pm 1.9$	$0.58 \pm 0.03$	$1.09 \pm 0.09^{**}$	8

The values represent mean  $\pm$  s.e. The Wilcoxon signed rank distribution was used for statistics  
Data are significantly different from Day 1 \* $p < 0.01$  \*\* $p < 0.05$



*Treatment of data* The results obtained from three successive light stimulations each day were pooled. Pupillary diameter was measured but the results were expressed as pupillary area in sq mm. Reduction in pupillary size after light stimulation was estimated. The time for half maximal contraction and dilatation of the pupil was determined and was used as a parameter for the speed of contraction and dilatation. The Wilcoxon signed rank test was used for statistical significance.

## Results

The most prominent observation in this study was that the half time of dilatation increased by 50% on day 5 compared to day 1. The half time of contraction was however unaffected. During the combat course a small reduction

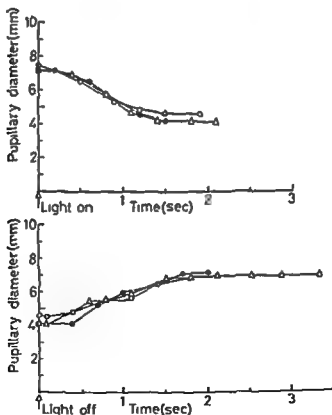


Fig 1

A typical example (cadet 8) of the pupillary response to the three successive light stimulations (top contraction bottom dilatation) before the course started (Day 1)

in pupillary size ( $A_1$ ) was observed. The effect was more pronounced on day 3 than on day 5. Pupillary size after light stimulation was unaffected during the course ( $A$ ). The reduction in pupillary size after light stimulation ( $A_1 - A$ ) was most pronounced on day 1 but the differences were only significant between day 1 and day 3 and between day 3 and day 5 (Table 1).

Five out of 9 cadets had small amplitude pupillary oscillations with a frequency of 0.2 sec on day 3 and day 5 which was never observed on day 1. A typical example of the pupillary response to light stimulation is given in Fig. 1.

## DISCUSSION

This study was done during a ranger combat course under field conditions necessitating the use of relatively simple instruments for the pupillographic studies. The military program of the combat course was on a very tight time schedule not allowing enough time for complete dark adaptation which would have been desirable. The test conditions on day 1 and 5 were very similar so that the differences observed between these two days are most probably due to severe combined stress the cadets had received. In contrast to this on day 3 the cadets were rapidly transferred from bright sunshine to the darkness of the examination room. The changes observed on day 3 compared to day 1 and day 5 may therefore in part be due to the different conditions under which the examination took place.

On day 5 the pupillary size was reduced by 14% compared to day 1 in agreement with previous work demonstrating that lack of sleep is accompanied by reduced pupillary size (Yoss et al. 1970). On day 3 however pupillary size was reduced even more (21%) and this may in part be due to incomplete dark adaptation. The following discussion will therefore be restricted to the differences observed between day 1 and day 5. The small amplitude pupillary oscillations observed on day 5 but not on day 1 have also previously been described following lack of sleep (Yoss et al. 1970) and may be interpreted as an imbalance between the sphincter and dilator tonus of the pupil.

The most prominent observation in this study was however that the pupil dilated much more slowly towards the end of the course whereas the speed of contraction was unaffected. This observation suggests that the dilator function of the pupil is more sensitive to prolonged combined stress than the sphincter function and this may in part be the explanation for the pupillary oscillations.

The test subjects were exposed to a severe combined stress of physical strain, sleep deprivation and caloric deficit. It is therefore from this study impossible

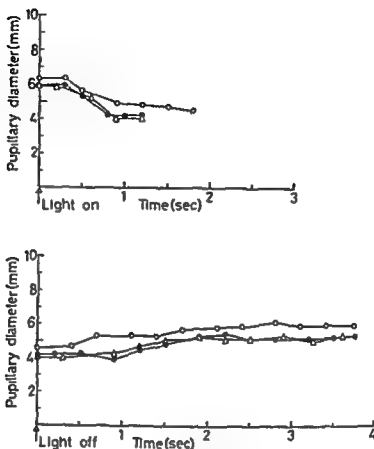


Fig 3

Pupillary response to the three successive light stimulations (cadet 8) at the end of the course (Day 5) top concentration bottom dilatation Note the pupillary oscillations at the end of the dilatation period and the slower dilatation of the pupil

to say which of these factors were responsible for the differences observed As for the underlying mechanisms the interpretations would be purely speculative and are therefore omitted

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# RELATIONSHIP BETWEEN BLOOD FLOW VELOCITY IN THE CHOROID AND INTRAOCULAR PRESSURE IN RABBITS\*

BY

I. TAKÁTS and F. LEISZTER

By using thermistors for calorimetry to monitor blood flow velocity in the choroid of the anaesthetized rabbit it was found that an elevation of intraocular pressure reduced the choroidal blood flow. The relationship between the ratio of perfusion pressure to intraocular tension ( $x$ ) and the reciprocal value of the percentage of blood flow velocity ( $1/y$ ) showed a curve convex towards the pressure axis. On a log lin scale the graph was linear. A significant linear correlation with a coefficient of 0.963 was obtained. The regression equation was found to be  $\log 1/y = 0.44x - 1.94 \pm 0.29$ . From the present experiments it is concluded that the vascular bed of the choroid in the rabbit is a passive one with no sign of autoregulation. The observations are discussed from the point of view of blood supply and possible damage to the optic disc.

*Key words:* choroid - vascular bed - blood flow velocity - ocular pressure - perfusion pressure - passive circulation - autoregulation - calorimetry - rabbit

It is common knowledge that in the dynamics of fluid circulation - as in electrodynamics - three factors are active: 1) the volume of the circulating fluid (intensity), 2) differential pressure (voltage) and 3) resistance of flow (resistance). The relationship of the three factors is  $\Gamma = \frac{IP}{K}$ . The application of this

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relationship (Hagen Poiseuille law applicable to Newton's fluid) to blood circulation in a closed system of pipes makes several considerations necessary. *Viz*  $R$  is directly proportional to the viscosity and length of the pipe and inversely proportional to the product  $r^4$ . The vascular wall is however elastic and the blood contains corpuscles etc. Yet with certain reservations about the capillary system this law may be applied to the circulation of blood. As compared with other parts of the body certain differences which concern the intraocular vascular system first of all that of the choroid should be emphasized. The diameter of the capillaries of the choroid is three times that of the usual. The pressure in the small arteries is relatively high (Duke Elder 1926 Bill 1963 Weigelin & Lobstein 1963 Takats 1970). Since the arterioles in the choroid are only short stumps the pressure in the capillaries is remarkably high. It follows from what has been said that the blood supply to the eye and particularly to the choroid is the highest in the body.

A further characteristic is that the pressure exerted by the tissue on the vessels is the highest in the eye they are under a pressure of 15–20 mmHg. From this it follows that the pressure in the venules has to be at least as much for the blood to be able to leave the eye. At the same time there is a great fall in pressure in the veins passing through the sclera. Extraocular venous pressure is 4–5 mmHg lower than intraocular pressure (waterfall phenomenon).

In the past decades interest has been focussed on the relationship between intraocular pressure, i.e. perfusion pressure and choroidal circulation as it has been proved that the prelaminar region of the optic nerve head is supplied mainly by centripetal branches from the adjacent peripapillary choroid (Hayreh 1972). It is known from investigations by Goldmann & Blok (1971) that the choroidal vessels are particularly sensitive to a rise in intraocular pressure. The latter authors applied mild pressure to the eyes of normal persons in response to which the blind spot was found to enlarge.

A knowledge of the relationship between intraocular pressure, i.e. a fall in perfusion pressure and choroidal blood flow is of great importance for the damage to the optic nerve in glaucoma. It is questionable if the vascular system of the choroid is capable of some autoregulation to compensate for the rise in intraocular pressure.

The aim of the investigations to be described was to determine the relationship between perfusion pressure and blood flow through the choroid in the case of pathological values of intraocular pressure. Similar examinations which however yielded different results were carried out by Niesel (1962) Friedman (1970) Alm & Bill (1973) and Armaly & Araki (1975).

## Materials and Methods

A total of 26 New Zealand albino rabbits were used. The animals were anaesthetized with urethane (1.0–1.5 g/b wt) injected into the marginal ear vein. To ensure good respiration a polyethylene tube was inserted into the trachea. Blood flow velocity was determined calorimetrically with a heated and a non-heated thermistor as described previously (Takats 1973). The thermistors were each introduced into a glass capillary, one of whose ends had previously been sealed up. After incising the conjunctiva the glass capillaries were fixed with 6/0 silk sutures to the temporal part of the sclera immediately behind the equator. The two thermistors were 5–10 mm apart. In order to isolate them from their surroundings the thermistors were covered with moistened cotton wool. The palpebral fissure was closed with sutures. The leads from the heating manganine wire and those from the thermistors were connected to a Wheatstone bridge as described by Seylaz (1965). The changes in intensity of the electric current proportional to the blood flow velocity were recorded on an X-Y recorder (Videoton Hungary). The intraocular pressure was changed by means of a needle placed in the anterior chamber. The needle was connected to a reservoir filled with mock aqueous humour and a pressure transducer. A separate pressure transducer measured the mean arterial pressure through a cannula introduced into the left femoral artery, the changes being recorded by a kipp Zonen micrograph.

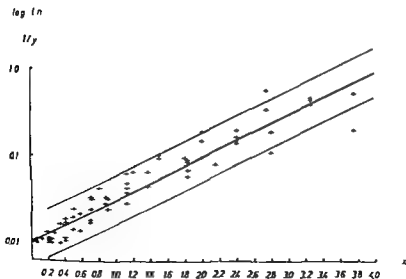
Blood flow velocity being directly proportional to blood flow through the tissue (Seylaz) it seemed justifiable to regard a decrease in blood flow velocity as a decrease in the volume of blood flow. According to the basic principle of the bridge blood flow velocity is directly proportional to the intensity of the electric current necessary for maintaining the difference in temperature between the two thermistors. This intensity of current on the other hand is directly proportional to the changes of the curve recorded by the recorder (e.g. 25 mA = 10 mm, 50 mA = 20 mm). The percentage changes in blood flow velocity were calculated from the changes of the curve recorded by the recorder, the base line corresponding to an intraocular pressure of 20 mmHg, which was taken as 0 per cent. The intraocular pressure was increased above the level of the systolic pressure in the femoral artery by raising the reservoir, as a result of which the choroidal circulation was stopped. The resulting change of the curve was taken as 100 per cent. The changes of the curve associated with the different values for intraocular pressure were expressed as percentages of the maximum deviation obtained in the manner described above. After resetting intraocular pressure at 20 mmHg a 10–15 min period of rest was allowed and then the intraocular pressure was increased stepwise 10 mmHg at a time by raising the reservoir until the change in the rate of choroidal blood flow c

## Results

The aim of the experiment was to determine the relationship between intraocular pressure and choroidal blood flow. Considering that there was a great difference in blood pressure between the animals, the perfusion pressure (mean femoral artery pressure minus intraocular pressure) was taken as one of the factors of our calculations. We had three values at our disposal: intraocular pressure, mean femoral artery pressure, and the percentage of the slowing of choroidal blood flow. The change in choroidal blood flow being a function of the intraocular pressure, the data obtained were plotted on a graph whose abscissa represented the perfusion pressure in relation to the intraocular pressure, which enabled a better approximation to the two values.

$$\frac{\text{mean femoral artery pressure} - \text{intraocular pressure}}{\text{intraocular pressure}}$$

On the ordinate were plotted the reciprocal values for the percentage of the slowing of choroidal blood flow. The diagram obtained in this way gave a curve convex toward the pressure axis.



*Fig. 1*

Reciprocal percentage of the slowing of choroidal blood flow ( $1/y$ ) and perfusion pressure in relation to the intraocular pressure ( $x$ ) on a log lin scale



In order to determine the exponent of the power function of the curve, a system of coordinates was used in which the logarithms of the above values were plotted on the ordinate.

Seventy six reliable experimental values could be represented in the graph. Using Gauss principle of the smallest squares a regression line was drawn, or to the points plotted in the logarithmic linear graph (Fig. 1). The correlation coefficient of this line was significant at the level of  $P < 0.001$ . The relation represented by the above mentioned function may be regarded as linear ( $r = 0.965$ ).

On the basis of our results the following interrelationship may be established between the ratio of perfusion pressure to intraocular pressure and the rate of choroidal blood flow:  $\log 1/y = 0.48x - 1.93 \pm 0.29$  ( $P$  = probability of 95%) where  $y$  = percentage of the slowing of choroidal blood flow,  $x$  = ratio of perfusion pressure to intraocular pressure.

## Discussion

In the present decade the investigations into the relationship between intraocular pressure and choroidal blood flow have not led to unambiguous results. Friedman (1970) who injected a solution containing krypton 83 into one of the long posterior ciliary arteries of a cat, drew the conclusion that the vascular system of the choroid behaved like a passive vascular bed up to 30 mmHg; above that level of pressure an active regulatory mechanism was brought into action. Weiter et al. (1975) using radioactively labelled microspheres 30  $\mu$ m diameter found that choroidal blood flow in the cat was stable up to 30 mmHg and that it was only above that value that it began to decrease linearly. On the basis of determining the amount of blood passing through one of the vortical veins Nakamura & Goolbsine (1973) found that above the level of 20 mmHg of intraocular pressure the rate of blood flow decreased in rabbits. From his examinations performed with a Doppler flow detector Tokoro (1972) reached the conclusion that below 25 mmHg blood flow velocity did not change; above that value the relationship between ocular tension and the decrease in blood flow velocity was linear. However, what he examined was the ciliary body and not the choroid.

In our own calorimetric examinations (Takats 1976) we also found that on increasing the intraocular pressure from 15 mmHg to 25 mmHg in rabbits choroidal blood flow began to slow. The investigation carried out by Alm & Bill (1973) with the help of radioactivity labelled microspheres 15  $\mu$ m in

diameter deserves attention. On increasing the intraocular pressure by 20.5 mmHg in monkeys the quantitative decrease in choroidal blood flow seemed linear. Using the heated thermocouple principle in cats and monkeys Armaly & Araki (1975) found that the choroid behaved like a passive vascular bed when the intraocular pressure was raised. The latter authors repeated Friedman's experiments and observed that the retrograde injection into the ciliary artery of a solution containing krypton 85 distributed the choroidal blood flow and led to erroneous results.

The present investigations also seemed to confirm the observation that in rabbits choroidal blood flow decreases linearly with decreasing perfusion pressure: no autoregulative mechanism manifested itself.

We are aware that the results obtained in rabbits should be viewed with reservation. The Seylaz method determines blood flow velocity and not volume of blood. Although as used under the conditions of the present investigation the method may be regarded as semiquantitative. Before our investigation Bill (1967) and Armaly & Araki (1975) who used a method which differed from ours but was still calorimetric, drew a similar conclusion as to the relationship between intraocular pressure and choroidal blood flow. It should also be taken into account that all these experiments, those of others as well as ours, were of an acute nature. The question may be raised whether a prolonged increase of 5-10 mmHg in the human eye does not call some regulatory mechanism into action. Clinical observations have not decided it unambiguously. According to several clinicians an intraocular pressure above the level of 22 mmHg continuing over a period of years results in damage to the optic disc. According to others in the case of the so-called ocular hypertension even 30 mmHg will not give rise to damage to the optic disc or to visual field defects (Koller & Becker 1977).

It may be that for moderate variations in intraocular pressure there is a regulatory mechanism which varies with the individual in range and vulnerability.

Within the limitations of the technique used, our investigations permit the conclusion that in response to an acute increase in intraocular pressure in the rabbit the vessels of the choroid show no sign of autoregulation of blood flow.

Only a non-invasive quantitative method applicable to man will enable final conclusions to be drawn. In this respect the method of Cristini et al (1975) may be regarded as an encouraging step. There is hope that by further increasing the sensitivity of the method the question of whether or not there is a regulatory mechanism in the choroid for moderate increases in intraocular pressure may be decided.

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## PILOCARPINE MEDICATION IN OPEN ANGLE GLAUCOMA

### A Study Using Pilocarpine Eyedrops and an Ocular Therapeutic System

BY

■ BRINCHMANN HANSEN and ■ ANMARKRUD

Two modes of topical pilocarpine therapy were applied to sixteen patients with open angle glaucoma. A pilocarpine drop medication (2% solution four times daily) was compared to a constant and continuous supply of pilocarpine by a therapeutic delivery system (Ocuser P 40). The pressure reducing efficiency was studied in the morning and in the following four hour period of an ordinary drop medication regimen.

The results indicate about equal pressure reducing properties in both high and low pre treatment pressure values. However compared to pilocarpine drops we found a statistically significant lower pressure in the morning after the Ocuser unit especially in glaucomatous eyes with high pre treatment pressure.

*Key words:* open angle glaucoma - pilocarpine - Ocuser - intraocular pressure

For a century (from 1874) pilocarpine has been administered by eye drop solutions to patients suffering from different kinds of glaucomas. In clinical usage a topical instillation is applied to the glaucomatous eye in a pulsed manner of medication usually four times daily during the waking hours of the patient. This mode of ocular self medication has proved itself very useful in reducing the intraocular pressure. Failure to get an expected pressure lowering response from drop medication could either be the result of inefficiency of the prescribed therapy or it could be the result of poor patient compliance (Armaly & Rao 1973).

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An ocular therapeutic system (Ocuser P 40) worn in either the upper or lower cul de sac will supply a glaucomatous eye with pilocarpine molecules at a constant rate of 40  $\mu\text{g/h}$ . This continuous release of drug has been shown to reduce the intraocular pressure substantially with less total amount of pilocarpine applied to the eye when compared with the pulsed dose medication (Armaly & Rao 1973). In the last few years papers have been published comparing drops and membrane units with regard to dose effect relationship both in normal eyes (Place et al 1975) and in hypertensive/glaucomatous eyes (Armaly & Rao 1973, Macoul & Pivan Langston 1975, Quigley et al 1975). These works show that the membrane controlled delivery system and the pilocarpine eyedrops achieved hypotensive results both in normal and open angled glaucomatous patients. Quigley et al (1975) found that a 40  $\mu\text{g/h}$  unit controlled eyes requiring 2% and 4% pilocarpine solutions four times daily. Other writers have been dealing with the Ocuser unit in other respects: patient compliance (Friederich 1974), side effects (less miosis and less myopia) (Brown et al 1976) and 24 h studies of effects of constant drug delivery to the glaucomatous eye (Armaly & Rao 1973).

The purpose of this study was to compare the two modes of topical pilocarpine medication in open angle glaucomas with regard to the pressure reducing efficiency. We wanted to study the effect on the intraocular pressure in the morning at a time just before a glaucomatous eye would receive a pilocarpine drop. We also wished to compare the effect on IOP during the following 4 h.

### Material and Methods

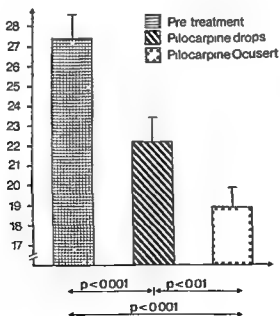
This study included sixteen patients with open angle glaucoma, all of them had previously been treated with pilocarpine in drop solution and all were responsive to this drug. They were free from external eye diseases and none had undergone surgical eye intervention. All of them had earlier been diagnosed as having glaucoma. The patients were included based on following criteria: intraocular pressure equal to or greater than 22 mmHg measured by Goldmann applanation in the sitting position and glaucomatous cupping of the optic disc with or without nerve fiber damage. A Haag Streit lamp and mydriatics were used to reveal pseudoexfoliation of the lens capsule. All of the patients were examined just prior to the study.

Pseudoexfoliation of the lens capsule was found in eight patients. There were 10 men and six women and the average age was 66 years (53-79). Number of eyes with glaucoma was 26. As a pre study regimen five eyes used epinephrine (four patients), two eyes used acetazolamide (one patient) and five eyes used

epinephrine and acetazolamide (three patients) The patients who used epinephrine and/or acetazolamide continued the same medication throughout the study

The mean pressure of three readings were used no measure varying more than 3 mmHg from the others The measurements were done by alternating between the patient's right and left eye

Each patient was subjected to two experimental treatment sessions Each session was preceded by a 44-48 h period during which time any pilocarpine medication was discontinued In the middle of this period the pressure was read at 9 a m in order to establish pre treatment pressure values In the one session the patients started a drop medication with one drop four times during one day (9 a m 1 p m 5 p m and 8 p m) The IOP was measured the next morning at 11 a m After this reading the patients were given by us one pilocarpine drop topically and the IOP was again measured at 11 a m and 1 p m In the other session an Ocuser unit was placed in either the upper or lower cul de sac at 9 a m and the IOP was measured the next morning at the



*Fig 1*

Mean  $\pm$  SEM in 25 eyes at 9 a m Significant differences between treatments given by P values

same hour of the day. The patients continued wearing the unit and the IOP was again measured at 11 a.m. and 1 p.m. The sequence of these sessions was randomly assigned for each patient.

## Results

All sixteen patients completed the study. They were well instructed how to replace the Ocusert unit in the lower cul de sac if the unit should fall out. We started out with 26 eyes; five eyes lost the unit during the 24 h trial time. The unit were at once replaced with the exception of one eye; thus 25 eyes completed the study.

The Student's *t* test (with  $P < 0.05$ ) was used in the statistical calculations.

Table I

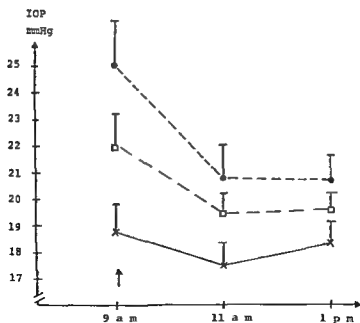
Mean IOP values during a four hour period in the two groups and in all patients after pilocarpine drops and Ocusert 1-40 respectively

		Pilocarpine drops			Ocusert P 40		
		9 a.m.	11 a.m.	1 p.m.	9 a.m.	11 a.m.	1 p.m.
a)							
Group I	Mean	18.9	17.5	18.33	17.4	15.1	19.94
IOP < 26 mm Hg	SEM	1.03	0.54	0.75	0.55	0.56	0.9
n = 12	Significance	3.5	2.92	2.61	1.97	3.01	3.37
		— n.s. — — n.s. —			— n.s. — — n.s. —		
		— n.s. —			— P < 0.05 —		
b)							
Group II	Mean	20.05	20.79	20.10	20.36	20.51	20.65
IOP ≥ 26 mm Hg	SEM	1.64	1.25	0.59	1.63	1.4	1.49
n = 13	Significance	5.93	4.50	3.21	6.03	6.75	5.33
		— P < 0.005 — — n.s. —			— n.s. — — n.s. —		
		— P < 0.005 —			— n.s. —		
c)							
Group I and II	Mean	22.05	19.45	19.56	18.94	19.6	20.33
	SEM	1.15	0.75	0.6	0.94	0.92	0.59
n = 2	Significance	5.19	3.15	3.12	4.14	4.9	4.46
		— P < 0.005 — — n.s. —			— n.s. — — n.s. —		
		— P < 0.005 —			— n.s. —		

**The effect on applanation pressure level**

Fig 1 gives the mean IOP reducing efficiency (measured at 9 a.m.) of the eyedrops and the unit in all 25 eyes. Both modes of pilocarpine administration reduced the pre-treatment pressure by a statistically significant amount ( $P < 0.001$ ). The protection against pressure rise in the morning however was greater using the Ocusert unit during the sleeping hours than the carrying over effect of the drops. This difference was statistically significant at a level  $P < 0.01$ .

In Table I a, b we did split the material in a high and a low pre-treatment pressure group. We chose IOP = 26 mmHg as a suitable level and we wanted to study the pressure reducing efficiency of the unit and the drops during a four hour period (9 a.m. - 1 p.m.). Figs 2 and 3 point out the advantage of using the constant drug delivery system especially in a group with high pre-treatment intraocular pressure. In the lower pressure group the carrying over effect of the drop medication seems to keep the IOP down to an acceptable level.



**Fig 2**

The effect on IOP during the four hour period after a single dose of pilocarpine drops (at 9 a.m.) (†) (mean  $\pm$  SEM)

x — x = group I    ● — — — ● = group II and □ — — □ = both groups



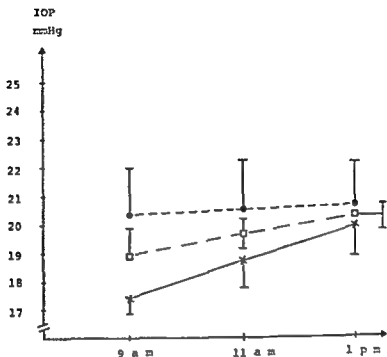


Fig 3

The effect on IOP during the four hour period during continuous use of the Ocuser unit

x — = group I ● - - - ● = group II and □ - - □ = both groups

After having received the morning pilocarpine drop both the Ocuser and the drop medications are about equal in their pressure lowering capabilities during the next four hour period

### Comments

Studies dealing with diurnal variations of the intraocular pressure (in both normal and glaucomatous eyes) seem to agree upon the importance of controlling the IOP especially during the morning and early part of the waking hours (Henkind et al 1973 Kitazawa & Horie 1975 Worthen 1976). These workers used frequent measurement during 24 h periods and found peak diurnal intraocular pressures in most subjects in the morning and early daytime. Our IOP readings were done in the morning at a time just before glaucomatous eyes would usually receive pilocarpine in drop medication. This critical

morning period is probably the least protected against pressure rise of all periods during the waking hours of patients with open angle glaucomas

Davanger (1964) has pointed out by use of hydrodynamic laws that the effect of miotics (in normal and glaucoma simplex eyes) increases in a systematic manner with increasing pressure and Krill & Newell (1964) point out in a clinical trial that the higher the average pre treatment tension the greater is the reduction produced by any strength of pilocarpine

This study confirms the efficiency of both the unit and the drops in reducing the intraocular pressure in open angle glaucomas Both modes of topical pilocarpine administration seem to have about equal pressure lowering properties in both high and low pre treatment pressure groups

The study also shows that the carrying over efficiency of pilocarpine drop medication during the sleeping hours is considerably less than the efficiency of the ocular therapeutic system used here The degree of insufficiency in reducing the morning IOP prior to the first pilocarpine drop of the day seems to increase with increasing pre treatment pressure level

A membrane controlled delivery system seems to be a better topical pilocarpine regimen compared to drop solutions in the treatment of open angle glaucoma with high pre treatment intraocular pressure

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## CAPILLARY HAEMANGIOMA OF THE OPTIC DISC

### A Case Report

BY

P GILBERT NIELSEN

A case of capillary haemangioma of the optic disc is reported. The ophthalmoscopic and fluorescein angiographic characteristics of the different varieties of haemangioma of the optic nerve head are discussed and the problems of treatment by photocoagulation outlined.

*Key words* haemangioma - optic disc - fluorescein angiography - photo coagulation

Angiomatosis retinae and von Hippel's disease are terms used synonymously to describe capillary angiomatous hamartomas of the retina and the optic nerve (Gass 1974). The development of an angioma in the optic disc or juxtapapillary retina is very uncommon. This report documents with the help of fluorescein angiography a case of capillary haemangioma of the optic disc. The various types of angiomas of the optic nerve head are tabulated and the problems of management outlined.

### Case Report

A 24 year old woman complained of blurred vision in the left eye some two months prior to being referred to the University Eye Clinic Odense in November 1976 for examination. No previous eye symptoms. There was nothing unusual in her past medical history. A younger sister suffers from epilepsy of unknown aetiology.

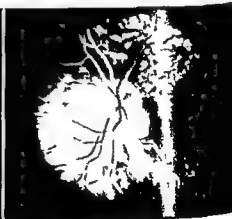
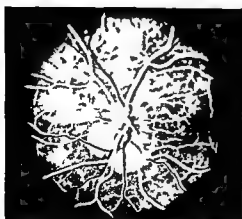
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*Fig 1*

November 1966 Reddish slightly elevated tumour on the left optic disc with cystic detachment and exudation Visual acuity 6/6



*Figs 2 and 3*

Fig 2 Angiogram arterial phase showing tumour vessels arising from the optic disc

Fig 3 Late angiogram showing massive dye leakage

### *Capillary Haemangioma of the Optic Disc*

Visual acuity was 6/6 emmetropic in both eyes. Right eye was normal. Fundoscopic examination of the left eye revealed a reddish tumour arising from the lower half of the optic disc and adjacent peripapillary retina. A double disc sized cystic detachment extended downwards. Discrete yellowish exudates at the margins of this detachment were noted. Perimetry showed enlargement of the blind spot and a paracentral scotoma, this finding corresponding well to those of the ophthalmoscopy. The intraocular tension was normal.

Fluorescein angiography demonstrated in the retinal arterial phase a well circumscribed mass of small calibre vessels confined to the area around the lower margin of the optic disc. Intraretinal leakage of the dye from the tumour was seen in the intermediate arteriovenous phase and late angiograms showed massive dye leakage. The calibration of the large central vessels was normal and no evidence of pigment epithelial or retinal abnormality outside the im-



*Fig 4*

July 1977 Central exudation and cystic detachment progressing. Note photocoagulation marks round the periphery. Applications on tumour vessels not visible.

mediate area of the slightly elevated optic nerve head tumour could be observed.

Some progression of the detachment and the exudation were noted during repeated follow up examinations over the next six months. Visual acuity remained 6/6 and the visual field defect was stationary. She was admitted for a general thorough evaluation in February 1977. No neurologic or other abnormality was found. Visual acuity in the left eye had become reduced to 6/9 by June 1977. Further progression of the detachment was noted and the exudation had increased with central oedema and the development of a macular star figure.

She was subjected to three applications of argon laser photocoagulation between June and November 1977. The photocoagulation was aimed directly onto the tumour vessels and round the periphery of the lesion. After a temporary improvement in visual acuity to 6/6 and apparently some regression in the affection the situation again deteriorated with a gradual reduction in visual



*Fig.*

April 1978. Complete macular star developed. Visual acuity reduced to 6/36.

acuity to 6/36 and the development of a complete macular star figure in January 1948. The visual acuity was unchanged and the exudation still progressing when last seen in April 1948.

### Discussion

Three varieties of haemangioma of the optic nerve head have been noted (Henkind & Benjamin 1946)

a) The racemose haemangioma is quite rare but may be part of the Wyburn-Mason syndrome with associated midbrain lesions or occurs as an isolated entity. The retina usually shows almost no clinical reaction, no haemorrhage, oedema or exudation. The bizarre vessel configurations may approach the macula very closely without interfering with visual acuity. Fluorescein angiography is helpful in demonstrating the abnormal arteriovenous connections. Quiescent cases show no vascular fluorescein leakage (Henkind et al. 1941).

b) The cavernous haemangioma is often asymptomatic and appears to be identical to intraretinal cavernous haemangiomas. The condition is not related to von Hippel-Lindau's disease. The fluorescein angiographic appearance is characteristic with the vascular spaces filling very slowly and often with a fluid level. No dye leakage occurs (MacDonald et al. 1945).

c) The capillary haemangioma range in appearance from small well-circumscribed elevated lesions obscuring part of the disc to large epipapillary vascular masses. Bilateral cases of papillary haemangiomas have been reported (MacNair et al. 1966) but on the whole the condition seems to be almost invariably unilateral. The usual type of retinal haemangiomas is bilateral in 50% of the cases (Duke Elder 1967). Unlike retinal haemangiomas, dilatation and tortuosity of the feeder vessels do not occur or are not visible in those angiomas arising from or immediately adjacent to the optic nerve head and angiographic evidence of arteriovenous shunting is difficult or impossible to demonstrate (Gass 1944). Johnson et al. (1946) found in an ultrastructural study the endothelial cells of the capillary haemangioma to be fenestrated, thus providing an explanation of the fluorescein leakage and the extravasated exudate that is characteristic of this tumour. About one quarter of patients with capillary haemangiomas have other clinical evidence of von Hippel-Lindau's disease (Duke Elder 1967). Visual loss occurs in more than half of the cases of capillary haemangioma and is generally the result of chronic macular oedema. Vitreous haemorrhage and retinal detachment may also occur (MacDonald et al. 1945).



Photocoagulation is the treatment of choice in retinal haemangiomas but for obvious reasons a risky venture when dealing with papillary just papillary lesions. The risk of destroying useful central vision is obvious particularly in those located on or surrounding the temporal margin of the optic disc. Photocoagulations of selected portions of the tumour may be successful in temporarily reducing the degree of intraretinal and subretinal exudation. The long term prognosis for central vision in these papillary and peripapillary tumours is however poor and photocoagulation should not be done in patients with normal visual acuity (Cass 1974). Repeated photocoagulations have not been successful in arresting the exudative changes and vision has decreased from 6/6 to 6/36 over the relatively short period of observation in the reported case.

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## HELICOIDAL PERIPAPILLARY CHORIORETINAL DEGENERATION

BY

KRISTJÁN SVEINSSON

A family with helicoidal peripapillary chorioretinal degeneration is described. This is a rare bilateral fundus affection - only seven more or less typical cases have been reported in the literature. 21 patients from the same family in four generations were examined: 10 men and 11 women. Seven men and six women showed a helicoidal affection. Of this number there were 11 children aged from 4-17 years, six were girls of whom three had helicoidal fundus and five were boys of whom two were affected. General examination revealed nothing of particular interest. We have here a congenital hereditary fundus anomaly or minor malformation in four generations - young people with normal visual acuity who develop with age a clear tendency to invasion of the macular region by a degenerative process. This is most dangerous for the visual acuity when the atrophic helicoidal wings lie in or near the macula region.

*Key words:* congenital hereditary fundus anomaly - bilateral peripapillary atrophy (Star fish atrophic wings)

In 1939 the author wrote an article in *Acta Ophthalmologica* (Sveinsson 1939) about curious fundus symptoms which he called chorioiditis arcata. Later he realized that this terminology was not correct as there was no inflammation. In this article the author describes four cases aged from 4-29 years with a curious fundus affection. Included in this is a 29 year old mother and her 4 year old son. The fundus showed peripapillary chorioretinal atrophy with more or less wide tongue shaped extensions to the periphery having no con-

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nection with the retinal vessels. In none of these cases did the atrophic ring affect the macular region. No oedema, haemorrhage or inflammatory process was observed. The pigment epithelium had disappeared in the helicoidal zones. A similar variety of fundus has since been described by others and named differently, e.g. circumpapillary dysgenesis strati pigmenti retinae (Rubio 1940, 1 case), Striate chorioretinitis (Kraffell 1955, 1 case) and Helicoid peripapillary chorioretinal degeneration (Franceschetti 1962, 1 case). As seen above the names of this fundus affection differ greatly. Franceschetti has more than anyone else investigated aberrant fundus affections. Therefore in this article the name suggested by him, although a long one, will be used. It is likely that it may often prove difficult to find correct and appropriate terminology for rare diseases or pathological anomalies when everything seems to have been described and classified. In many cases there are gaps in our knowledge and in this curious affection there does not appear to be either an inflammatory process or a degeneration but rather a congenital anomaly or a minor malformation. Helicoidal fundus changes are rare. Franceschetti (1967) states: "In fact we have found seven more or less typical cases in the literature which were presented under different names. These cases all describe individuals. However the author has found four cases including mother and son. Hence it was of interest to follow up and carry out research on the family (mother and son) originally reported in 1939."

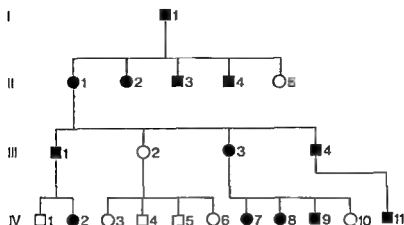
Nearly 40 years have elapsed since then and four generations (21 individuals) come now to be analyzed. It should therefore be possible to discover whether we are dealing with a hereditary affection or not. In Franceschetti's remarkable book *Chorioretinal Heredo Degenerations* he writes about helicoidal chorioretinal degenerations and states: "We have, however, still no proof that the infantile form of helicoidal peripapillary chorioretinal degeneration is congenital and stationary. In this article it is intended to point out that the cases observed in the family in question are congenital hereditary fundus anomalies or minor malformations — apparently stationary infantile forms with a normal visual acuity."

With advancing age these patients develop degenerative changes mostly in the central region — endangering the central vision. This is of course most marked when the wings (the tongue-like radiating atrophies) lie in or near the macula.

## Results

Examinations have been carried out on 21 persons spanning four generations, aged from 4–70 years (Fig. 1).

# *Helicoidal Peripapillary Degeneration*



*Fig 1*

Genealogical tree of a family with dominant transmission of a helicoidal peripapillary chororetinal degeneration through four generations (by Kristjan Sveinsson)

□ = male - normal

○ = female - normal

■ = male helicoid degeneration

● = female helicoid degeneration

## **I Generation**

The ancestor of this family was examined in 1935 (his wife was dead). He was then 10 years old and beginning to see rather poorly. He had peculiar chororetinal foci in both fundi but he died before further examinations could be performed.

## **II Generation**

Five of his children were available for examination. One daughter, A. T., 68 years old (II-1) is the woman examined and reported in 1939 - and also her son, then four years old. The woman had widely spread helicoidal changes in fundi which lay very close to macula in both eyes. Daughter number two, 63 years old (II-2) had helicoidal fundus vis 6/6 in both eyes. Both sons had very clear helicoidal fundus changes. One son, 61 years old (II-3) had poor vision in one eye because of ablatio retinae and helicoidal fundi. The vision in the other eye was 6/12 with pigmentary changes in the macula. The other son, 54 years old (II-4) had very poor vision; the helicoidal changes were very evident and the macula area was affected by helicoidal foci. Daughter number three, 51 years old (II-5) had normal fundi and vision 6/9 but incipient pigmentary changes were present in the macula in both eyes.

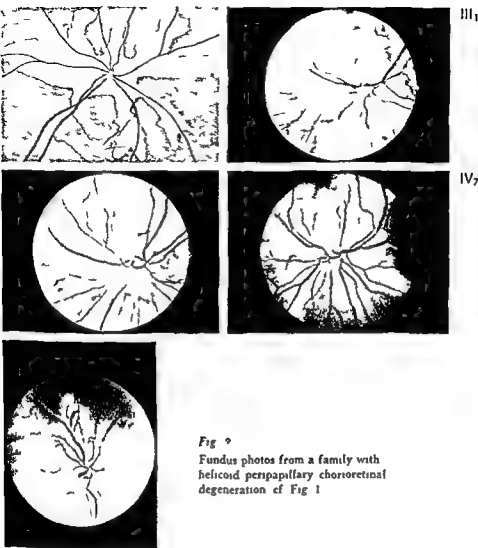
### III and IV Generation

The children of woman A T (II 1) are as follows Son 43 years old III 1, the one examined when he was four years old with a helicoidal fundus His condition is now vis 6/6 in both eyes but there are primary signs of pigmentary changes in the central region with a mottled appearance both papillae are normal His two children a son 21 years old IV 1 has a normal fundus and his daughter 19 years old IV 2 has inherited her father's fundus (macula normal) Daughter 3, years old III 2 she and her four children IV 3 4 5 and 6 have a normal fundus Daughter III 3 33 years old has a helicoidal fundus + three of her four children two daughters IV 7 and 8 and son IV 9 all have a helicoidal fundus daughter IV 10 has normal fundus son 28 years old III 4 and his son IV 11 both have helicoidal fundus daughter IV 12 has normal fundus son 29 years old III 4 and his son IV 11 both have helicoidal fundus

### Fundus examination

These helicoidal fundus changes are similar in all the younger members of the family The description refers to those younger family members with both eyes affected Normal vitreous structure well coloured distinctly defined optic disc Fundus peripapillary atrophy spreading from which can be seen narrow or wide tongue shaped wings extending into the periphery and seemingly reaching further into the nasal retina area In some areas their edges are sharp whereas in other areas smaller or larger notches are observed Choroidal veins can be clearly seen in the periphery and at the edges of these wings Elsewhere the shapeless white scleral area can be seen over the most part of the wings where the pigment epithelium has almost or completely disappeared Spreading of slight pigmentation is sometimes to be seen over these spots The retinal vessels which in appearance seem to be quite normal lie over these spots in some areas without following them

In most of the young people the fundus is otherwise normal except in some areas close to the edges adjoining the atrophic wings where the colour is a rather darker red It is therefore probable that the pigment layer that should have covered the atrophic spots could not reach any further and has accumulated into a double layer It could also partially be a contrast phenomenon against the white area Niveau changes do not seem to exist Oedema exudates and haemorrhages are not found in these fundi Vision is nearly normal in most of the young people a few have a mild astigmatism Serious myopia does not occur Visual field for white is normal but defects appear in the visual field corresponding to the fundus damage Colour vision Stilling Ishihara adjustment is normal Dark adaptation is normal



*Fig 9*

Fundus photos from a family with helicoidal peripapillary chorioretinal degeneration cf Fig 1

This description of the fundus applies to both young and old persons. However, with age the young patients start to develop a degenerative stratum pigmentum retinae chiefly in the central region of the eye.

The patient A T 68 years old II 1 (Fig 2) has been followed up for the longest period. At the time of the first examination in 1936 she was 26 years old and widely spread helicoidal changes were found in both fundi very close to macula.

Vision was then 6/12 in the right eye with - 2.0 sph and 6/9 in the left eye with - 1.0 sph. When the patient was close to fifty years of age she lost the central vision and was then only able to count fingers eccentrically in the periphery of the eye. The atrophic process had greatly progressed in the macular and peripapillary regions. Since then the fundus anomalies have been stationary. Both brothers have developed reduced vision. A few years ago the elder brother 61 years old II-3 had 6/9 vision in the right eye but this has now fallen to 6/12 with pigment changes in the macula. He lost sight in the left eye after an ablation of the retina and had helicoidal fundus changes. The younger brother II-4 54 years old has always experienced difficulty in reading because of the extensive helicoidal fundus changes. It is remarkable that the fault in the pigment epithelium near the edges of the atrophic wings is more noticeable in the central region of the eyes in all three siblings. The 43 year old son of A. T., III-1 seems to be developing a degeneration of the pigment epithelium in the central region.

### Discussion

Twenty one persons were examined 10 men and 11 women as shown in Fig. 1. The four generations - seven men and five women were found to have a congenital helicoidal fundus affection. Of this number there were 11 children aged from 4-17 years six girls of whom three had a helicoidal fundus and five boys of whom two were affected. This is therefore a clear hereditary case with an autosomal dominant mode of transmission. Dr. L. Magnusson an ophthalmologist in Akureyri has come across one family living in North Iceland where this curious alteration of the fundus occurs in many of the family members. In all likelihood this is the same family.

What could be the cause for this strange fundus anomaly? From a genetic point of view this anomaly or malformation is clearly distinct from other hereditary affections of the fundus. Many sclerotic diseases occur in the choroid which can cause atrophy and destruction of the pigment epithelium e.g. in such cases as atrophic choroidal sclerosis and choroidal atrophy. These diseases often appear with massive pigmentary proliferation which is spread over the fundus and is then progressive. This occurs more often in adults and is therefore not similar to helicoidal affection. Judging by the shape of the atrophic fundus spots one could suggest insufficiency or defects in one or more branches of the ciliary arteries ciliares posteriores breves which would not only cause defects in the choroid, but would also cause secondary damage in the pigmentary epithelium and retina. This is not a case of inflammatory or obvious degenerative disease.

fection in young people but represents either a congenital inherited anomaly or a malformation resulting from a development disorder in the formation of the chorioidea or stratum pigmenti retinae or lack or defect or aplasia in arteriae ciliares posticae breves. An autosomal dominance is here the usual mode of transmission.

All hereditary diseases of course depend initially on the development of a pathogenic gene from a normal gene. The members of this family who are apparent carriers of the same pathological gene present a similar clinical picture and gene expression. The gene has a high penetrance and a high degree of expression and in these cases the pathological gene is expressed only in part of the tissue that it is generally associated with.

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## AXIAL LENGTH OF THE EMMETROPIC EYE AND ITS RELATION TO THE HEAD SIZE

BY

JON S. LARSEN

The results of the ultrasonographic measurements of the ocular components in 40 emmetropic eyes from men of nearly the same age are presented and the possible relation between the axial eye length and the head size is discussed. A significant correlation was found between the axial eye length and the head circumference ( $P < 0.001$ ), between the axial eye length and the head length ( $P < 0.01$ ) and between the axial eye length and the head breadth ( $P < 0.02$ ). A significant correlation was also found between the axial eye length and the height of the subjects ( $P < 0.01$ ).

It is suggested that such a correlation can explain not only the wide individual range of axial eye lengths in emmetropic eyes but also the existing difference in axial eye length between the sexes.

The mean eye length was 23.82 mm in 40 men and 23.02 mm in 10 women, both sexes were of the same age (20-30 years).

*Key words:* ultrasonography - axial eye length - emmetropia - head size.

The average value of the axial length of emmetropic eyes is given in more recent investigations as lying between 23.2 and 23.5 mm (Gernet 1964, 1966, François & Goes 1971, 1974, Karantinos et al 1974). In the majority of the studies a considerable variation in axial length of emmetropic eyes is found (Gernet 1964, 1966, Fridman & Savitskaya 1966, François & Goes 1971) and this can be more than 5.0 mm (François & Goes 1971). There seems also to be agreement that the axial eye length is on average greater for men than for women (Gernet 1964, Nover & Grote 1965, Fridman & Savitskaya 1966, Larsen 1971, François & Goes 1977).

A review of the literature on this topic does not appear to disclose any information which can explain the difference in axial length between the sexes or the individual variation. No studies seem to have been done either to reveal to what extent there exists a correlation between the head size and the axial length of the emmetropic eye. The object of this work was therefore to further clarify these relationships by examination of emmetropic individuals of the same age and sex.

## Material

The material included 50 emmetropic eyes (refraction between  $-0.5$  and  $+0.5$  dptr) of subjects between 20 and 300 years of age. All the subjects 40 men (average age 26.4 years) and 10 women (average age 24.7 years) had 20/20 vision in the right eye which was examined.

## Methods

The head length was measured between the most projecting part of the back of the head (opisthocranium) and glabella. The head breadth was measured between the two most protruding points above *porus acusticus externus*. The head circumference was measured all round the most projecting part of *squama frontalis* and the most projecting part of *os occipitale*.

All measurements of the eye were performed under cycloplegia. Cyclopentolate hydrochloride 1% (Cyclogyl®) was instilled twice at intervals of about 10 min 30–45 min before a streak retinoscopy and the examinations were carried out. The Javal Schiotz ophthalmometer (Haag Streit) was used to determine the corneal radius. The results were the average value between the horizontal and vertical radius. The horizontal corneal diameter was determined from a colour photograph (magnitude at *limbus corneae* 1:1).

The depth of the anterior chamber (including corneal thickness), axial thickness of the lens and the vitreous length were measured ultrasonographically. The ultrasound apparatus used in the research consisted of an A scan instrument (Kretztechnik model 7200 MA) and of a 10 MHz/5 mm transducer (NM 10/5 k). Small swim goggles with perforated glasses were employed for the immersion technique. In order to avoid echographic disturbances from cilia and eyelids a small blepharostat (model Barraquer) was used. The echograph was calibrated in microseconds and the calibration checked before each new subject was examined. In order to calculate the ocular distance in millimeters we used the following ultrasound velocities: 1532 m/sec in the camera anterior and the vitreous and 1641 m/sec in the lens (Jansson & Löck 1969). The distance of the ocular parameters were measured from a polaroid photo with a precision compass (Helios) including an odometer graduated in 1/20 mm. The equipment setting and amplification were those used for obtaining a maximal amplitude echo of a detached retina. An average of five measurements with an optimum re-

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## AXIAL LENGTH OF THE EMMETROPIC EYE AND ITS RELATION TO THE HEAD SIZE

BY

JON S. LARSEN

The results of the ultrasonographic measurements of the ocular components in 40 emmetropic eyes from men of nearly the same age are presented and the possible relation between the axial eye length and the head size is discussed. A significant correlation was found between the axial eye length and the head circumference ( $P < 0.001$ ) between the axial eye length and the head length ( $P < 0.01$ ) and between the axial eye length and the head breadth ( $P < 0.01$ ). A significant correlation was also found between the axial eye length and the height of the subjects ( $P < 0.01$ ).

It is suggested that such a correlation can explain not only the wide individual range of axial eye lengths in emmetropic eyes but also the existing difference in axial eye length between the sexes.

The mean eye length was 23.52 mm in 40 men and 23.0 mm in 10 women both sexes were of the same age (20-30 years).

*Key words:* ultrasonography - axial eye length - emmetropia - head size.

The average value of the axial length of emmetropic eyes is given in most recent investigations as lying between 23.2 and 23.5 mm (Gernet 1964 1967 François & Goes 1971 Karantinos et al 1974). In the majority of the studies a considerable variation in axial length of emmetropic eyes is found (Gernet 1964 1967 Fridman & Savitskaya 1966 François & Goes 1971) and this can be more than 5.0 mm (François & Goes 1971). There seems also to be agreement that the axial eye length is on average, greater for men than for women (Gernet 1964 Nover & Grote 1965 Fridman & Savitskaya 1966 Larsen 1971 François & Goes 1977).

**Table I**  
Correlations between the eye length the various head dimensions  
and the subjects height

No and sex of subjects	40 Males
	Axial eye length
Head circumference	$r = +0.6055$ $t = 4.6599$ $P < 0.001$
Head length	$r = +0.4103$ $t = 3.9851$ $P < 0.01$
Head breadth	$r = +0.3157$ $t = 2.4953$ $0.01 < P < 0.02$
Height of subjects	$r = +0.4451$ $t = 3.0690$ $P < 0.01$

We found a significant correlation between the head circumference and the axial eye length ( $P < 0.001$ ) Fig 1 Table V. There was also a significant correlation between the head length and the axial eye length ( $P < 0.01$ ) between the head breadth and the axial eye length ( $P < 0.02$ ) and between the height and the axial eye length ( $P < 0.01$ ). In this material of 40 men there was an important correlation between the head circumference and the height of the subjects ( $r = +0.5555$   $P < 0.001$ ).

### Discussion

The relationship between the different ocular parameters found in this investigations (Table III) was in agreement with findings from former studies of emmetropic eyes. Between the corneal radius and the axial eye length Sorsby et al (1957) obtained a correlation coefficient of  $r = +0.817$  and François & Goes (1971) determined a correlation factor of  $r = +0.64$  in emmetropic eyes. Between the depth of the anterior chamber and the axial eye length François & Goes (1971) determined a correlation factor of  $r = +0.4634$ . We

obtained also a rather important positive correlation between the corneal radius and the axial eye length ( $r = +0.4569$ ) as well as between the depth of the anterior chamber and the axial eye length ( $r = +0.5424$ ). We measured a significant negative correlation between the depth of the anterior chamber and the thickness of the lens ( $r = -0.2808$   $P < 0.05$ ). Other authors could also demonstrate an important negative correlation between the depth of the anterior chamber and the thickness of the lens in emmetropic eyes (Delmarcelle et al 1969 1970  $r = -0.60$  François & Goes 1977  $r = -0.5110$ ). In our study the axial length and the thickness of the lens varied independently ( $r = -0.1200$   $0.20 < P < 0.50$ ). François & Goes (1971 1977) after comprehensive studies of emmetropic eyes also came to the same conclusion. Our material seems therefore although it was relatively small to be representative with respect to the relationship between the ocular components.

We could demonstrate a rather important difference of 0.80 mm between the mean eye length in men (23.82 mm) and in women (23.02 mm). Other results in the literature could also demonstrate a longer eye length of 0.60–0.80 mm in men as compared to women (Gernet 1964 0.60 mm Nover & Grote 1965 0.60 mm Fridman & Savitskaya 1966 0.71 mm Fridman 1968 0.70 mm François & Goes 1977 0.80 mm). The authors agree that the variations between the eye lengths in emmetropic eyes are considerable and can be more than 0.0 mm (Gernet 1964 3.55 mm Fridman & Savitskaya 1966 3.1 mm Franceschetti & Luyckx 1967 3.0 mm François & Goes 1971 0.9 mm present study 3.09 mm (men)).

In the literature there seems to be no information which can explain these circumstances. We could however in this investigation demonstrate a significant correlation between the axial length in emmetropic eyes and the head size. It is suggested that such a correlation can explain not only the wide individual range of axial eye lengths in emmetropic eyes but also the existing difference in axial eye length between the sexes.

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## REFRACTION IN ADULT WEST GREENLAND ESKIMOS

### A Population Study of Spherical Refractive Errors Including Oculometric and Familial Correlations

BY

P H ALSBIRK

*Refractive error (RF)* was determined using subjective technique and Snellen chart in a West Greenland Eskimo population in the district of Umanaq. Both eyes of 433 and one eye of 25 persons were refracted in age groups above 15 years. Spherical equivalents of any astigmatic errors were included in RF values.

*Emmetropic excess and myopic skewness* of RF distributions were found. A significant trend towards hypermetropia with age was found in women, not in men. Myopia was no rarity: 63% of male eyes and 42% of female eyes showed myopia stronger than -2.0 diopters. Moderate myopia -5 to -1 d was found in 13.3% and higher myopia in 0.8% of the persons.

*RF and ocular dimensions* showed strong correlation for axial and vitreous length ( $r = -0.45$ ) only and weaker correlations for chamber depth and thickness of lens and cornea. No association was found between RF and height.

*Family variations of RF* were compared with those of ocular dimensions. Judged by child-parent relationships a much lower heritability was found in RF ( $h = 0.14$ ) than in axial length, corneal curvature and chamber depth ( $h = 0.76, 0.64$  and  $0.56$  respectively). However, sibs showed a higher RF similarity than child-parent relationships, suggesting an influence of common familial environment upon RF, although theoretically genetic (dominance variance) factors may also be responsible.

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Recent epidemiologic and genetic studies strongly emphasize the importance of environmental factors to human refraction. The present family study supports such ideas. Refraction seems to be highly influenced by environment in spite of the mainly genetic determination of relevant ocular dimensions.

**Key words:** refractive error - myopia - ocular dimensions - height - age/sex - Greenland Eskimos - heritability - genes/environment

Through most of this century since the days of Steiger (1913) the majority of human refractive errors have been considered to be genetically determined. As late as 1972 Sorsby expressed his concept of refraction based on several studies by the following words: 'The traditional emphasis on environmental factors as productive of refractive errors finds no support in the detailed studies of to day. As to low myopia developed during growth Goldschmidt (1968) like most other modern authors shared this opinion. However he pointed out that a rare late myopia exists which is caused by extensive fatiguing near work.

In fact the many low or simple myopes of civilized populations were considered to result from relaxed natural selection in such communities (e.g. Post 1962) against the nearly non existing myopia in primitive populations. Thus the surveys of Holm (1937) in Gabon Negroes and Skeller (1954) in pure East Greenland Eskimos were taken to support a genetic hypothesis.

However this mainly genetic concept of refractive errors has not been unopposed through the last decades e.g. Lindner (1944). In Japan the old European theories of school myopia have currently received strong support although the pathogenetic mechanism has been the object of much dispute, e.g. Sato (1957) and Otsuka (1967). Recently epidemiologic and genetic surveys in North American Eskimos have shown that the traditional genetic concept of refractive errors has to be modified. An adolescent epidemic of myopia has been independently reported in Alaskan and Canadian Eskimos by Young et al (1969), Morgan & Munro (1973) and Alsbirk & Forsius (1973). Furthermore a number of family studies have shown that the heritability of refractive error is low, about 25% of the survey by Spry (1976) based on reports by Sorsby et al (1966), Young & Leary (1972) and Hegmann et al (1974) as well as the Eskimo family study by Morgan et al (1975).

The present population study of refractive error in adult Greenland Eskimos contributes to the epidemiology and genetics of this important visual parameter. Relevant oculometric findings are included.



## Material and Methods

The basic material of the present study was 931 Greenland Eskimos living in a district of Umanaq in 1969. They constituted 95% of the two following census population groups: 612 town inhabitants above 15 years and 278 persons in the 8 villages aged 40 years or more (Alsbirk 1974). This sample was examined by *optical pachymetry* in 1969. The adults aged 15 years or more were reexamined in 1972 using *ultrasound ophthalmometry* and in the summer of 1972 using *Javal Schiøtz keratometry* and *subjective refraction* in front of Snellen's chart at 6 m. cf. Alsbirk (1974). The refractions were made in cooperation with ophthalmic assistant Ida Alsbirk who has several years' experience in prescription of glasses in Greenland.

At the *refraction survey* one village (Satut with 20 persons) was excluded due to lack of time. Out of the remaining 120 adults 533 were examined. Apart from persons absent (31), moved (53), dead (29) or diseased (4) only 40 persons (= 7%) of the target group were not examined, due to non appearance (32) or refusal (8).

Corneal and subjective astigmatism will be described in a later publication. In the present paper the *refractive error (RE)* is given in spherical equivalents, i.e. sphere +  $\frac{1}{2}$   $\times$  signed cylindrical refractive error. When possible the average of both eyes was taken to represent the individual. Cycloplegia was not used, as we were not prepared to do angle closure glaucoma surgery possibly indicated in some shallow chamber angle individuals of this high risk population (Alsbirk 1976).

Thus RE was estimated in 50% of the 533 persons examined in both eyes of 11 and in one eye of 25 persons. A total of 73 eyes (14%) could not be refracted, mostly due to poor visual acuity or lack of cooperation. Visual acuity distributions including rate of blindness as well as use and need of spectacle corrections will be described elsewhere.

## Results

*Distributions of refractive errors (RE)* are illustrated in Fig. 1. The well known unimodal non-Gaussian pattern was found ( $P < 0.001$  by  $\chi^2$  goodness of fit tests). An excess around emmetropia was found in both sexes but a test of kurtosis was significant in men only ( $P < 0.001$ ). In both sexes a significant myopic skewness was found:  $P < 0.02$  in men and  $P < 0.001$  in women.

*Age variation of RE* was insignificant in men but showed a positive regression coefficient in women in whom an increase of 1.2 diopters from age 20 to 70 was found (cf. Table 1 and Fig. 2). Thus a highly significant sex difference appeared in persons more than 40 years old, the women being 0.3 d more hypermetropic than men. In younger generation an opposite insignificant trend was found.

RF of right and left eyes could be compared in the 483 persons of Table I. No significant side difference was found (average 0.02 d). Right and left eyes showed a high mutual similarity (correlation coefficient  $r = +0.85$ ). Thus about 90% of all persons refracted showed anisometropia below 1.0 d, 95% below 1.5 d and 99% below 3 d while only 4 persons had higher anisometropia about 5 d.

Rates of myopia are given in Table I as percentual frequencies according to two simple arbitrarily chosen principles of truncation. About 6% of male and 4% of female eyes showed myopia stronger than  $-2.0$  d (95% confidence intervals: Men  $6.3 \pm 3.2\%$ , women  $4.2 \pm 2.5\%$ , each person counted once).

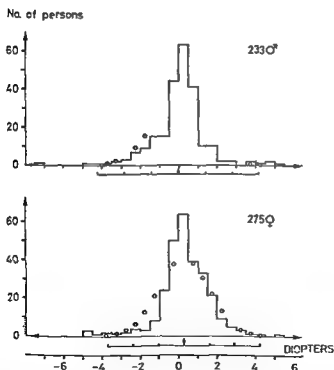


Fig 1

Refractive error (RF) distributions of adult Greenland Eskimos based on 991 eyes of 503 persons. Spherical equivalents are given, averaged in bilaterally refracted persons. The mean values  $\pm 3$  sd are shown below the histograms. The circles indicate expected nos. per half diopter class according to a Gaussian distribution.

Table I

Refractive errors (RF) in 453 Greenland Eskimos bilaterally refracted according to age sex and percentage of myopic eyes

Age	No and sex of persons	RF as average of both eyes (diopters)				Myopia in per cent RF more negative than	
		mean	sd	median	range	-2.0 d	0.0 d
15-19	20 M	-0.4	1.7	0.0	-3.5 to +3.0	15	45
20-29	31 M	+0.1	0.6	+0.1	-1.5 to +2.0	-	97
30-39	51 M	+0.2	0.9	0.0	-2.4 to +3.5	21	94
40-49	53 M	-0.5	1.6	0.0	-7.4 to +2.6	94	43
50-59	55 M	-0.0	1.4	-0.2	-2.5 to +4.1	26	55
60+	57 M	-0.2	1.4	+0.1	-4.0 to +2.0	108	46
15-39	94 M	+0.0	1.06	0.0	-3.5 to +3.0	43	99.31
40+	123 M	-0.18	1.50	-0.1	-7.4 to +4.1	78	45.4
15+	222 M	-0.0	1.34	0.0	-7.4 to +5.0	63	49.31
15-19	29 F	-0.2	1.0	-0.2	-2.5 to +2.5	17	45
20-29	37 F	-0.2	1.3	0.0	-4.5 to +1.5	81	39
30-39	45 F	-0.1	0.8	0.0	-2.4 to +1.5	42	41
40-49	56 F	+0.2	1.0	+0.3	-5.0 to +1.8	18	34
50-59	43 F	-0.5	1.6	+0.5	-5.0 to +3.6	58	29
60	45 F	+1.0	1.6	+1.2	-4.5 to +5.0	42	15
15-39	114 F	-0.14	1.01	0.0	-4.5 to +2.5	48	49.11
40	141 F	+0.55	1.43	+0.5	-5.0 to +5.0	51	96.2
15+	261 F	+0.25	1.30	+0.25	-5.0 to +3.0	42	35.13

Sex variation: 1)  $\chi^2 = 3.1$  n.s. 2)  $\chi^2 = 13.7$   $P < 0.001$  3)  $\gamma = 2.4$  n.s.

Age variation estimated by linear regression coefficient (b)

In 222 M  $b = -0.004$  diopters per year  $s_b = 0.006$  n.s.

261 F  $b = +0.024$  diopters per year  $s_b = 0.005$   $P < 0.001$

Frequencies of all negative RF values are included in Table I for the purpose of comparison with Young et al (1969) Alaska Eskimos showed the following per cent of myopes age 16-20 59%, 21-25 88%, 26-30 43%, 31-40 23%, 41-50 4%, and 51 and above 0% (right eyes only cycloplegically refracted). In the Canadian Eskimo survey (Morgan & Munro 1973) moderate myopia was taken as 1 to 3 diopters of myopia. In the present survey we found  $-5.0 \leq \text{RF} \leq -1.0$  d in 94%, 9%, and 1% of the pooled age groups 15-19, 20-29 and 30-39 years respectively (average 12.0%), above 40 years the rates 11%, 19%, and 14% were found in age groups 40-49, 50-59 and 60+ years (average 14.2%). The fairly high estimate in 15-19 year old

adolescents is based on only 12/49 persons but approximates the value given by Moran & Munro (1973) about 29%. High myopia RF more negative than -5.0 was found in one eye of 11 men (-10 and -5.5 d) and in one eye of 11 women (-6.2 and -5.0 d) i.e. in one eye of 0.8% of the persons bilaterally refracted

RF and ocular dimensions could be correlated in a subsample of 323 persons in whom all oculometric variables had been measured (cf Alsibirk 1977). As the 137 men and 188 women showed nearly identical values pooled estimates are given. Between RF and ocular dimension the following correlation coefficients were found (in ranked list): Vitreous length -0.45\*\* axial length  $r = -0.44^{**}$  anterior chamber depth (age and sex independent deviation score) -0.18\* radius of corneal curvature +0.03 corneal thickness +0.12\* (cf Alsibirk 1978) lens thickness +0.15\* (-0.06 in men, +0.37\* in women). The asterisks indicate significance at the 0.05 or 0.01\*\* level. Thus well known more or less pronounced associations between ocular dimensions and RF were found.

RF and height could be analysed in a subsample of village inhabitants aged 43 years or more in whom height had been measured 9 years earlier by Littauer et al. (1976). Insignificant correlations were found  $r = +0.20$  in 66 men and  $r = +0.08$  in 63 women. Thus no association between body height and myopia was suggested in this group.

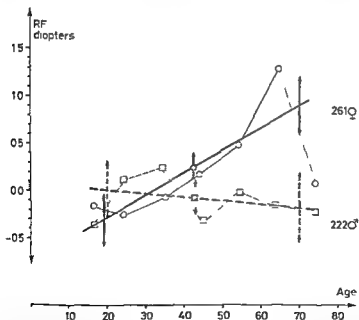


Fig. 9

RF and age in adult Greenland Eskimos based on 453 persons bilaterally refracted. Decade mean values (males  $\square$  females  $\circ$ ) and linear regressions with 95% confidence limits at mean ages and arbitrary extreme ages are shown.

Table II

Family variation of age and sex independent ocular dimensions: corneal radius of curvature (CR), axial length (AL), chamber depth (ACD) and spherical refractive error (RF). Estimates are given as regression coefficients ( $b$ ), intrapair correlation coefficients ( $r_1$ ) with standard errors ( $se$ ).

Family relationship	No of pairs	CR		AL		ACD		RF		Maxim. genetic expect.
		$b$	$se$	$b$	$se$	$b$	$se$	$b$	$se$	$b$
Child father	52	0.26	0.12	0.46	0.15	0.42	0.11	0.13	0.11	0.5
Child mother	107	0.36	0.09	0.34	0.11	0.21	0.08	0.03	0.09	0.3
Child parent	159	0.32	0.07	0.33	0.09	0.23	0.07	0.07	0.06	0.5
Child midparent	49	0.47	0.17	0.55	0.22	0.74	0.18	0.02	0.17	1.0
Husband wife	103	0.02	0.10	0.05	0.10	-0.09	0.10	0.03	0.09	0.0
		$r_1$	$se$	$r_1$	$se$	$r_1$	$se$	$r_1$	$se$	$r_1$
Brothers	29	0.27	0.18	0.40	0.19	0.53	0.13	0.39	0.16	0.5
Sisters	51	0.29	0.13	0.19	0.14	0.40	0.12	0.27	0.13	0.5
Sibs	160	0.20	0.03	0.25	0.07	0.43	0.06	0.25	0.0	0.5

Family variations of RF and ocular dimensions are shown in Table II. Due to age and sex variation of RF a transformation of dioptric error to age and sex independent deviation score was performed prior to analyses. For the purpose of comparison three relevant oculometric variables: axial length, corneal radius of curvature and anterior chamber depth were transformed correspondingly. Detailed procedures are described elsewhere (Alsbirk 1975).

The subgroups of the family material showed conspicuous variations which, however, must be considered on the background of the large standard errors, even in the pooled groups of Table II.

Husband wife pairs showed no mutual similarity and thus no assortative mating seems to occur. First degree relationships showed largely the same fairly high level of familial oculometric resemblance in child-parent as well as in sib comparisons. In contrast to this pattern the refractive error analyses showed no significant child-parent correlation but a significant sib correlation. Although the standard errors call for reservation, the finding suggests that certain influences upon RF tend to make sibs more alike in refraction than children and parents. Two explanations seem possible: common familial environmental factors during childhood influencing adulthood refraction or a genetic factor, the so-called dominance variance component. Interpretation will be discussed below.

## Discussion

Recent changes of refraction in Eskimos has been a challenge to traditional mainly genetic concepts of the human refractive state. The stability through generations of refractive error distributions has now lost global validity at least in rapidly changing communities. In the *Alaska and Canada Eskimo* surveys the elderly adults had grown up as arctic nomads in small hunting communities without schooling while after world war II children and adolescents as a rule have attended school regularly. The present study was made among *West Greenlanders* living in a hunting and fishing community. The development through this century has run a fairly quiet course here compared to the violent changes in Alaska and Canada. West Greenland has been under Danish administration for about 250 years. For more than 100 years a written Eskimo language has been taught in schools and analphabetism is practically eradicated although the general population does not read much. All adult age groups of the Umanaq Eskimos were found to be fairly often myopic. According to earlier clinical observations this should not be a great surprise (Bertelsen 1940, Lawætz 1949, Skeller 1949) although population studies have been lacking. On the other hand the isolated Angmagssalik district in *East Greenland* has a special historical background. The population here was colonized as late as 1894 and no written East Greenland language exists, their dialect being very unlike that of the West Greenland communities. It has now been made completely clear that the very rare myopia found by Skeller (1954, no eye more myopic than  $-1.25$  d among 1100 eyes examined) is not a general or permanent characteristic of all Eskimos.

The present study did not include children and was made without cycloplegia. At least two additional studies ought to be done in Greenland in the near future: a repeated investigation of Skeller's material from Angmagssalik where much has changed since 1950, now supplemented with a new generation. Further a cycloplegic population survey in children and adolescents of one of the greater towns (and school centers) of West Greenland in order to see if there is an adolescent epidemic of myopia in such more rapidly developing communities.

Surprisingly the age variation differed between men and women in the present study. The hypermetropic trend with increasing age corresponds to general clinical experience of Slataper (1950) but was found in women only. A recent population study in Israel revealed a similar preponderance of females among hypermetropes and of males among low myopes above the age of 40 (cf. Hyams et al. (1971)). Socioeconomic factors were not included in the present analyses. However in a subsample of men having some education (16 teachers, clerks, telegraphists) no significant excess of myopia was found.

*Family variations of refractive error* and its determining oculometric variables have only been analysed in a few earlier studies. The preponderance of genetic factors in European concepts of refraction has largely been based on selected pedigrees and twin studies but important sources of bias have rarely been recognized. As the present analyses show, the methods of quantitative genetics are useful but at least as crude as the standard errors are large. However, important family studies recently published agree with the present trends. Thus Hegman et al (1974) found a low refractive error parent-offspring resemblance in their randomly selected material of 181 families which gave a heritability estimate of  $h^2 \pm sr = 0.24 \pm 0.05$ . Thus a low heritability of spherical refractive errors was found compared with e.g. interpupillary distance ( $h^2 = 0.65 \pm 0.05$ ) or corneal power ( $h^2 = 0.89 \pm 0.05$ ; Mash et al 1975). Based on the present child-parent regressions of Table II, heritability of refractive error was estimated at  $h^2 = 0.14 \pm 0.12$  while relevant ocular dimensions showed higher values: corneal curvature radius  $h^2 = 0.64 \pm 0.14$ , axial length  $h^2 = 0.76 \pm 0.18$  and anterior chamber depth  $0.56 \pm 0.14$ .

A relatively higher resemblance between sibs than between parent and offspring was a prominent finding in the present refractive error analyses and similar results appeared in Sorsby et al's study (1966) and particularly in the Eskimo family study performed by Young et al (1969). On the other hand, sibs from Washington State studied by Young (1953) showed a low mutual resemblance (partial  $r = 0.14$ ). Such discrepancies are not easily explained. Although dominance variance through the sharing by sibs of one quarter of all pairs of alleles may be a significant source of higher resemblance in sibs, environmental influences bound to the family conditions may also exist. Such *common familial environmental factors* may be strong in some and weak in other populations and periods. Twin studies especially may give such inflated levels of resemblance which will only be recognized if several monozygotic twins reared apart can be analysed and this is very rare. Probably sibs and half sibs would be more useful in an attempt to separate such common familial environment and dominance effects in human genetics (cf. Li (1971)).

However, in spite of such uncertainties it is now clear that the conclusions based on twin studies like that of Sorsby et al (1962) have not been confirmed neither by their own (1966) nor by other larger studies of family variations of refractive error. The static genetic concept of refraction has to be modified. Once again environmental factors probably related to near visual demands of school age seem to be important (e.g. Tscherning 1883; Lindner 1944; Sato 1957; Morgan et al 1975; Young 1971). As a clinical consequence of such changing views, Oakley & Young (1975) treated a group of 226 slightly myopic children with +1.5 d bifocal add to distance correction. Compared with the

annual progression of  $-0.5$  d in the control group the bifocally treated children showed a negligible average progression ( $-0.03$  d per year). A few other reports have appeared e.g. Bedrossian (1961) who successfully used atropine monocularly to stop myopic progression and Kelly et al (1965) who achieved arrest of myopia using bifocals (add +1.5) and phenylephrine 5%. However such reports as well as the current views of Japanese ophthalmology have generally been received with great scepticism. Probably a new era reaccepting the importance of environmental influence in myopia pathogenesis is now at the beginning due to results of epidemiologic, genetic and experimental research through the last ten years.

In conclusion the present study has shown that myopia is fairly frequent in all adult ages in West Greenland Eskimos who have all attended school and use a written language. No adolescent epidemic of myopia was found in the district population examined. However age variation showed a sex difference, elderly women being more hypermetropic than men. Family variations in adults showed almost no resemblance between the refraction of children and parents but a somewhat higher correlation between sibs. The results are cautiously interpreted as a support to the redeveloped concepts of environmental influence upon human refractive state. Refraction shows a lower heritability than relevant ocular dimensions.

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# PREVALENCE OF PINGUECULA IN GREENLAND AND IN COPENHAGEN AND ITS RELATION TO PTERYGIUM AND SPHEROID DEGENERATION

BY

M S NORN

The prevalence of pinguecula has been studied by slit lamp examination of 659 Eskimos in South Greenland and 810 Caucasians in Copenhagen. The overall prevalence was found to be 26 per cent in Greenland against 41 per cent in Copenhagen ( $P < 0.001$ ) and to rise with increasing age though with a fall after the age of 60 among Greenland women. Pingueculae (measured by their vertical height) are largest among Greenlanders and larger in males than in females. They increase in size with increasing age.

They are generally located nasally in Greenlanders and temporally in Copenhageners.

The prevalence of pinguecula is almost 1½ times higher among Greenlanders than among Copenhageners while that of spheroid degeneration and that of pterygium are 3 times and slightly over 10 times higher respectively.

The incidence of pinguecula and spheroid degeneration are correlated per site and per subject in the two geographically different series.

Pterygium is not correlated with regard to site this being always located nasally. Pterygium practically never harbours spheroid degeneration neither in its body nor in its head (91 pterygia).

Pinguecula and pterygium are therefore to be regarded as two different disorders while spheroid degeneration is related to pinguecula.

*Key words:* cornea - sclera - pinguecula - pterygium - degeneration, spheroid, limbal, corneoscleral - Greenland Eskimos - Copenhagen Caucasians - prevalence - correlation

Pinguecula is a yellowish circumscribed slightly elevated pad like formation seen on the exposed part of the bulbar conjunctiva close to the cornea. It consists of connective tissue subjected to elastoid degeneration.

Many investigators believe pinguecula to be a precursor of pterygium.

The object of the present study has been to estimate the prevalence of pinguecula within two geographic territories. One is South Greenland where pterygium is a frequent disorder (8.6 per cent) and the other Copenhagen where pterygium is rare (0.7 per cent, Norn 1978b).

The report comprises recording of the three paralimbal degenerative processes (pterygium, pinguecula and spheroid degeneration) all characterized histologically as elastoid degeneration and probably all due to climatic factors, presumably ultraviolet light.

### Method and Material

All pingueculae of minimum 0.2 mm were recorded when disclosed by slit lamp examination with ten times magnification.

The statistical calculation was based on Student's *t* test.  $P < 0.05$  is regarded as significant.

The total series comprised 1469 subjects: 659 Eskimos examined in South Greenland (arctic territory with much ultraviolet light) and 810 Caucasians in a big city (Copenhagen with a temperate moist climate). The prevalences of pterygium and spheroid degeneration as well as the age and sex incidences of the series under review have been described previously (Norn 1978a, b).

### Results

Pinguecula was more frequent in Greenland than in Copenhagen. The overall prevalence per subject was 5.6 per cent among Greenland Eskimos against 1.1 per cent among Caucasians in Copenhagen ( $P < 0.001$ ).

Fig. 1 shows the age and sex curves. The prevalence of pinguecula rises with increasing age until a level is reached after the age of 60 years. However, among female Greenlanders the prevalence declines after the age of 60 years ( $P < 0.001$  in the age group of 40-59 compared with that of 60 or older).

A tendency was noticed towards preponderance of males, but the difference was not significant.

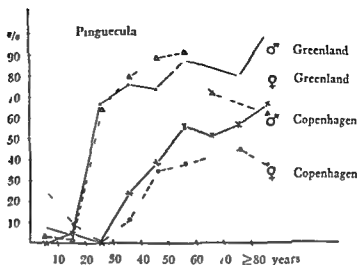


Fig 1

Prevalences of pinguecula among 659 Eskimos in South Greenland and 810 Caucasians in Copenhagen distributed according to sex and age

Abscissa 10 year age groups

Ordinate incidence of pinguecula

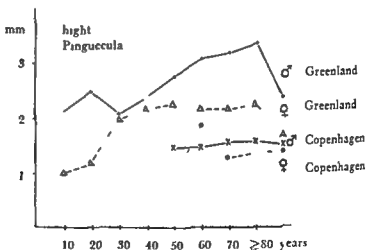


Fig 2

Size of pinguecula Dependence on age sex and geographic territory (Eskimos in South Greenland and Caucasians in Copenhagen)

Abscissa 10 year age groups

Ordinate Mean vertical height of pinguecula in millimetres

(The curves for young Copenhageners have been ruled out. The groups are too small)

In 43 per cent of the Greenlanders and 54 per cent of the Copenhageners the pinguecula was typically *triangular* with its base towards the cornea and its apex towards the inner or the outer canthus. In 7 and 6 per cent respectively the shape was horizontal oblong and in the remaining cases more or less circular.

The vertical height of the pinguecula was noticed to rise with increasing age being the highest in the Greenlanders and particularly high in men (Fig. 2).

Table I shows a significant difference with regard to sex between the Greenlanders and the Copenhageners. The vertical height averaged 2.43 mm in the Greenlanders and 1.48 mm in the Copenhageners.

Pingueculae above 3 mm in height were present in 21.8 per cent of the Greenlanders against 1.2 per cent of the Copenhageners ( $P < 0.001$ ).

In Greenland relatively large pingueculae (above 3 mm) were most frequent even in elderly individuals (in 26 per cent aged over 50 years compared with 7 per cent of the younger individuals  $P < 0.01$ ). In Copenhagen the age difference was not significant.

In Greenland the mean height of the pinguecula was 2.83 mm in males and 2.0 mm in females. In Copenhagen the corresponding figures were 1.61 and 1.40 mm.

The nasally located pingueculae are generally larger than those located temporally. In Greenland the average figures were 2.61 mm for the nasal and 2.11 for the temporal pingueculae. In Copenhagen the figures were 1.79 and 1.92 mm respectively.

The pingueculae have only been seen localized within the exposed part of the bulbar conjunctiva. Among the Greenlanders the pinguecula was more developed and most often located nasally (82/47) and among the Copenhageners most often temporally (53/46). The differences are statistically significant (Table II). In most cases it is located both temporally and nasally.

*Table I*  
Average vertical height of pinguecula. Sex difference among Eskimos in Greenland and Caucasians in Copenhagen.

	Men	Women	P
Greenland > 3.0 mm	30%	17%	< 0.001
Copenhagen > 2.0 mm	20%	12%	< 0.01

Table II

Size of pinnae in per cent of maximum possibilities. A total of 1000 pinnae (52 Greenlanders with pinnae = 522 and 478 temporal or nasal = 522 x 4 maximal sites - and 300 Copenhageners)

	Nasal	Temporal	P	Number of sites with pinnae subjected
Greenland	52 (50% - 44)	47 (30% - 44)	< 0.01	522
Copenhagen	45 (50% - 65)	30 (30% - 60)	< 0.01	300

Fig. 3 illustrates the distribution over sites in the two series. In Greenland binasal and all four possible sites (two nasal and two temporal in the ear individual) were the most frequent combinations. In Copenhagen binasal and mono temporal occurrence predominated. The Figure further indicates

Pinnae's %

Greenland	nasal		Copenhagen	Bilateral
13.6	○	○	13.9	0
30	◀○	○	19.6	(
25.5	○	◀○	9.9	4.6
35	◀○	○	6.2	(
11	◀○	◀○	1.9	0.6
30	◀○	○	17.9	1
12	◀○	◀○	3	0.3
5	◀○	○	11.7	1.3
25	◀○	◀○	15.1	4.3
100%			100%	100%

Fig. 3

Size of pinnae in per cent of maximum possibilities in Greenland and 410 Caucasians in Copenhagen (compared with a revision of Himmels series of 412 Caucasians from the Switzerland 1921). Whether the disorder has affected the right or the left ear has been considered, but attention has been focused on the number of pinnae and their distribution over nasal and temporal sites, a total of nine possible combinations

Table III

Interrelation of pinguecula and pterygium in the same subject (639 Greenlanders and 810 Copenhageners) The  $\chi^2$  test for two independent samples

	Greenland		Copenhagen	
	+ pteryg	- pteryg	+ pteryg	- pteryg
+ pinguecula	15	32	5	325
- pinguecula	12	25	1	49

$\chi^2 = 11.48 \quad P < 0.001$

$\chi^2 = 2.94 \quad P > 0.05 \quad n.s.$

the sake of comparison a calculation based on Hinnen's series from Basel in 1921

Pingueculae occur on an average at more sites in the eyes of Greenlanders than in those of Copenhageners

#### Relation to pterygium

Concurrence of pterygium and pinguecula at the same site was seen in no more than three cases (3.81 = 4 per cent)

Note however that a pinguecula might be concealed to be concealed or have been modified in the "body" of the pterygium thus being non detectable

In the Copenhagen series no correlation was found between pinguecula and pterygium, even if present in the same subject (in the same or the opposite eye) whereas a correlation was noticed in the Greenland series (Table III)

The pinguecula was no larger in the males with concurrent pterygium compared with males without pterygium (in the Greenland series the mean vertical pinguecula height was 2.82 mm in subjects with pterygium and 2.83 mm in the total series. In the Copenhagen series the corresponding figures were 1.59 and 1.61 mm respectively) The pinguecula was perhaps a little larger in the female Greenlanders with pterygium (2.69 against 2.20 mm) whereas it was smaller in the female Copenhageners (0.73 against 1.40 mm)

#### Relation to spheroid degeneration

The incidences of spheroid degeneration and pinguecula were correlated in the two geographically distinct series both per subject and per site (all



$P < 0.001$  Vorn 1962a) the majority of the spheroid globules having been found on the pinguicula.

This means that the spheroid globules may be characterized as mainly a pinguicula, a kind of spherical condensation of the elastoid degenerating the pinguicula.

If a pterygium represented a further development of a pinguicula mainly in the body of the pterygium, we should expect to find a frequent occurrence of spheroid degeneration on a pterygium.

This was not so in our material. In fact, in no more than three of the cases of pterygium was spheroid degeneration seen in relation to the disk. In the stated three cases spheroid globules were present on the cornea close to the head of the pterygium. Only in one case spheroid degeneration was observed on the body of the pterygium (Vorn 1962b).

## Discussion

Skeller in 1940 examined 309 individuals in North and West Greenland and reduced pinguiculae in 71 per cent of the males and 46 per cent of the females, i.e. prevalences corresponding to or being a little on the large side of the figures arrived at for South Greenland.

For boys under 10 years of age the percentage was 8 in Skeller's series compared with 71 in the present.

Like pterygium, pinguicula seems to be a very frequent disorder in Greenland (Clemmensen 1956). However, no further investigations have been published from Greenland.

Young & Firlay (1955) examined 929 individuals in Newfoundland and Labrador (Esquimaux, Peled Indians and Caucasians). They recorded prevalence and plotted age incidence curves on pinguicula corresponding approximately to the findings in the Greenland series under review. Note that the age incidence curve for females fell after the age of 70 years, while the incidence among males rose to nearly 100 per cent, all in conformity with the condition in the present Greenland series.

Forsius & Eriksson (1953) examined 709 individuals with a binocular slit lamp glass in Helsinki, Finland. They likewise noticed pinguiculae among males and a rising prevalence with increasing age, though perhaps with a fall among women aged over 70 years.

In addition, they found a higher prevalence among outdoor workers than among indoor workers. The criteria employed do not permit a direct comparison with the series under review, but the prevalences seem to correspond.

those found for Greenlanders (83-78 per cent among males and 78-50 per cent among females outdoor and indoor respectively)

In Basel Switzerland Hinnen in 1921 examined 462 individuals in Gull-Strand's slit lamp. He found the prevalence to rise with increasing age. The overall prevalence was 38 per cent. This finding corresponds approximately to the figures for the present Copenhagen series.

The great difference in prevalence between the Greenlanders and the Copenhageners is significant, the two series having been examined by the same person by the same procedure and within the same period.

The difference between nasal and temporal location is remarkable. Among the Greenlanders the pinguecula was most often located nasally and among the Copenhageners temporally.

All previous investigators found preponderance of nasal site for pinguecula. Young & Finlay (1975) in Canada found 611 nasal against 483 temporal. Hinnen (1921) in Basel 16 per cent nasal against 2 per cent temporal and 20 per cent at both sites (i.e. a total of 38 per cent).

The temporal preponderance in Copenhagen is thus exceptional.

Forsius & Eriksson (1962) on a rough grading of the size found the nasal pingueculae to be on an average larger than the temporal ones. This view was borne out in the present investigation by direct measurement of the vertical height both in Greenland and in Copenhagen.

Forsius (1972) likewise found the size of pinguecula to increase with increasing age. However the curves perhaps suggest a minor decrease at extremely high ages. Unfortunately the present Greenland series is too small to allow an estimate of a similar tendency in this series.

Pterygium is believed by many workers to develop from a pinguecula (Fuchs 1891, Hilgers 1960, Cameron 1965). This view is supported by their histological structures, both consist of hyaline connective tissue subjected to elastoid degeneration (Hogan & Zimmerman 1962, Hogan & Alvarado 1967, Klintworth 1972) and the raised prevalence within territories with much ultra violet light.

Others hold that these disorders have a common cause but that development of pterygium requires an extra releasing factor (Garner 1972, Young & Finlay 1975).

Friede (1949) mentions episcleritis and peripheral defect of Bowman's membrane as additional causes of development of pterygium.

Forsius & Eriksson (1962) found the pinguecula to be the largest in individuals with concurrent pterygium. This observation could not be borne out in the present series.

In 1963 Forsius & Eriksson declared that the incidence of pterygium among young individuals argues against its development from a pinguecula. They workers have also seen pterygia develop without the presence of a pinguecula doubtless due to a genetic factor.

The frequency of pterygium and band shaped climatic keratopathy are not correlated (Hawaii, Finland - Forsius 1968).

I have previously demonstrated a correlation between spheroid degeneration and pinguecula (Norn 1978a). Simultaneous examinations of the three degenerative processes in two geographically distinct series with different prevalences of pterygium gave occasion for certain reflections.

All three types of degeneration were found to be more frequent within the territory with much ultraviolet light and mainly outdoor work (South Greenland) than in Copenhagen.

Pterygium was ten times more frequent (8.6 against 0.7 per cent, Norn 1979), spheroid degeneration three times more frequent (12.3 against 4.1 per cent, Norn 1978a) and pinguecula scarcely one and a half times more frequent (5.6 against 4.1 per cent) in Greenland than in Copenhagen. In other words: an unequal distribution with a strikingly low incidence of pterygium among Copenhageners.

The prevalences of the degenerative processes rise with increasing age, though falls may be seen at very high ages both for pinguecula and spheroid degeneration, but this is hardly the case with pterygium. Pingueculae grow in size with increasing age, but not so pterygia.

The distribution between the nasal and temporal site is unequal. In the series under review, pterygium was always found nasally and never temporally. Pinguecula and spheroid degeneration were most often located nasally in the Greenland series (nasal/temporal pinguecula 82/47, spheroid degeneration 84/20) but temporally in the Copenhagen series (temporal/nasal pinguecula 58/46, spheroid degeneration 37/32).

Pinguecula and spheroid degeneration tend to spread in approximately the same direction within the two geographically distinct territories, whereas pterygium differs completely from these.

Spheroid degeneration may occur in a pinguecula. It may be characterized as a condensation of the elastoid degeneration of the connective tissue of the pinguecula.

If a pterygium represented a further development of a pinguecula, we should expect to find larger pingueculae in individuals with pterygium and to observe spheroid degeneration in the body of the pterygium. Such conditions were, however, not detectable by measuring the vertical heights of pingueculae and

- by close examination of pterygia in the slit lamp with ultraviolet and indirect white light
- 1 We may therefore be justified in regarding pterygium and pinguecula as two distinct disorders
- Spheroid degeneration is related to pinguecula but not to pterygium

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# THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

## 1 The Basal and Intermediate Layers of the Perilimbal Zone

BY

STEFAN LATKOVIC and SVEN ERIK G NILSSON

The present paper on the perilimbal zone of the guinea pig conjunctival epithelium describes the ultrastructural organization of the basal cell layer including basal epithelial cells melanocytes and small lymphocytes as well as the intermediate cell layers consisting of polyhedral cells and processes from the melanocytes and the small lymphocytes. Particular attention is paid to those cell features that might be associated with phagocytosis autophagy and transport mechanisms.

**Key words:** conjunctival epithelium - perilimbal zone - guinea pig - ultrastructure - transmission electron microscopy

In humans the most common eye diseases affect the conjunctiva. The majority of these diseases are infectious and/or inflammatory in nature. It may therefore be of general interest to study at the ultrastructural level the morphological changes as well as the mechanisms of defense of the conjunctival epithelium that occur in such diseases. Bacteria of different strains of *Shigella* (Racz & Tenner 1963) *Salmonella* (Tenner et al 1971) and *Listeria* (Zimanski et al 1974) have been found inside the conjunctival epithelial cells. In the case of *Listeria* where bacteria were observed intracellularly within membrane limited vacuoles the process was regarded as phagocytosis. In connection with *Salmonella* (light microscopy) the terms endocytosis and phagocytosis

cytosis were used. However the significance of the finding of intracellularly located bacteria in this as well as in other epithelia is still unclear and the question as to whether bacteria penetrate into the host cells or are phagocytized by them needs to be investigated further.

In an electron microscopic investigation to be published (Latkovic & Nilsson 1979b) we demonstrated uptake of latex microspheres a nonmetabolic, non-toxic marker by conjunctival epithelial cells indicating active phagocytic capability of these cells. An additional study concerning bacteria is in progress.

As a background for the investigations outlined above a knowledge of the architecture of the normal conjunctival epithelium and of the ultrastructure of its cells including the migratory cells was needed.

The aims of the present series of papers are (1) to give a detailed description of the ultrastructural organization of all morphologically different zones of the normal conjunctival epithelium as studied in a continuous strip of tissue from the limbus to the lid margin which has not previously been done and (2) to emphasize those morphological features that might be associated with the processes of endocytosis, autophagy and transport.

Most of the recent and/or more complete ultrastructural investigations of the conjunctival epithelium concern the perilimbal zone (Wanko et al 1964; Hogan et al 1971; Radnot 1971), the palpebral zone (Takakusaki 1969) or the tarsal zone (Dark et al 1974; Gremer et al 1977). A short report on bulbar conjunctiva was included in a study of ocular pemphigus (Carroll & Kuwabara 1969) and a very short survey of bulbar, fornical and tarsal conjunctiva was published by Weingeist (1973). In older studies in which specimens may have also been taken from the fornices the descriptions are generally less comprehensive and to a large extent concentrated upon certain cell organelles, cell types or intercellular relations (Suzuki 1956, 1957; de Toledo et al 1957; Shibuya 1958; Fujiyama 1961; Segawa 1962; de Toledo 1966).

## Material and Methods

Four clinically healthy adult pigmented guinea pigs weighing 300–500 g were used for this study. The animals were anaesthetized intraperitoneally with 50 mg/kg b.w. sodium pentobarbital (Nembutal® Abbott) and perfused (pressure 120 mmHg) via the left ventricle with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. Each eye was excised in one piece including the eyelids. Fixation was continued by immersion in glutaraldehyde solution as above but at +4°C during the next 2 h. 1–2 mm wide parallel sided strips of the conjunctiva were then cut, ex-

tending from the corneal periphery to the margin of the lower eyelid. Part of the abundant subconjunctival tissue was removed. After a rinse in 0.1 M sodium cacodylate buffer the strips of conjunctival tissue were post fixed in 1% osmium tetroxide in veronal acetate buffer at pH 7.2 for 1 h. Acetone in increasing concentrations was used for dehydration.

For light microscopy the tissue blocks were embedded in methacrylate cut in large sections at right angles to the limbus on a Porter Blum microtome and stained with toluidine blue. The light micrographs were taken as a series of overlapping negatives at the same magnification. The final micrograph represents a montage of exactly matched parts.

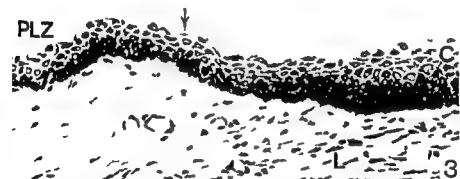
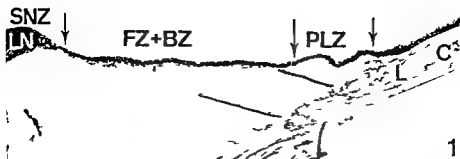
For transmission electron microscopy embedding was done in Vestopal W. Thin sections were cut on the LKB Ultratome parallel to the limbus. The sections were first placed on Formvar filmed grids coated on the back with carbon and then stained with uranyl acetate for 1 h and lead citrate for 5 min. The specimens were examined in a Philips EM 300 at an accelerating voltage of 80 kV (or in a few cases at 100 kV).

## Observations

### General aspects

The general appearance of the guinea pig conjunctiva is shown in low power light micrographs (Figs 1 and 2). It is composed of a stratified non keratinizing epithelium and an underlying stroma. The main part of the epithelium is three to five layered consisting of basal intermediate (polyhedral) and superficial epithelial cells as well as specialized cells (melanocytes goblet cells) in certain zones and occasional migrating cells. Towards the limbus and the lid margin the epithelium is more complex gradually changing into other types of epithelia (limbal corneal and epidermal). The stroma is composed of loose connective tissue blood and lymph vessels and a larger number of migrating cells than in the epithelium. A lymphoid nodule adherent to the overlying epithelium is located at about  $\frac{1}{3}$  of the distance between the limbus and the lid margin.

The conjunctival epithelium can be divided topographically into a number of zones (Figs 1 and 2) that will be described in four subsequent papers: the perilimbal zone (PLZ) in the present paper I (the deeper layers) and in paper II (the superficial layer) (Latkovic & Nilsson 1979a) the bulbar zone (BZ) the zone of the fornix (FZ) and the supranodular zone (SNZ) (overlying the lymphoid nodule) in paper III (Latkovic 1979a) the palpebral zone (PZ) and the perimarginal zone (PMZ) in paper IV (Latkovic 1979b). The limbal epithelium is not included in the present study since it does not belong to the conjunctiva proper. Except for the greater number of cell layers melanocytes and melanosomes (melanin granules) in the limbal epithelium the major differences between the perilimbal and the limbal epithelia were observed.



*Figs 1-3*

1 Low power light micrograph of the perlimbal zone (PLZ) the bulbar zone (B7) the zone of the fornix (FZ) and part of the supranodular zone (SNZ) of the conjunctival epithelium of the guinea pig C cornea L limbus LN part of the subepithelial lymphoid nodule  $\times 60$

2 Low power light micrograph of the supranodular (SNZ) palpebral (PZ) and perimarginal (PMZ) zones of the conjunctival epithelium LN lymphoid nodule LM lid margin  $\times 60$

3 Survey light micrograph of the epithelium of the perlimbal zone (PLZ) of the conjunctiva and of the epithelium overlying the limbus (L) The number of cell layers is greater in the latter than in the former epithelium The dark cells in the basal and suprabasal layers are rich in melanin pigment C cornea  $\times 3,0$





Fig 4

Survey electron micrograph from the perilimbal zone. A layer of superficial cells (S), intermediate layers of polyhedral cells (P) and a layer of basal cells (B) are seen. A melanocyte process (MP) extends into the suprabasal layer. Small lymphocytes (LY) with rather electron lucent cytoplasm are found between the basal and suprabasal cells. DP, dendrite like processes from lymphocytes or melanocytes. N, nuclei. M, melanosomes. CS, conjunctival stroma. Arrow points to the basal lamina.  $\times 4900$ .

### **The basal cell layer of the perilimbal zone**

The basal cell layer of the conjunctival epithelium was continuous with the same layer of the limbal and the corneal epithelium (Figs 1 and 3). This single cell layer was composed of basal epithelial cells, a certain number of melanocytes and occasional small lymphocytes (Figs 4-7). The basal lamina, separated from the epithelial cells by an electron lucent zone, followed closely the inner border of the basal cells (Figs 4, 5 and 7). The lamina was connected to the basal cells by half desmosomes (Fig 5).

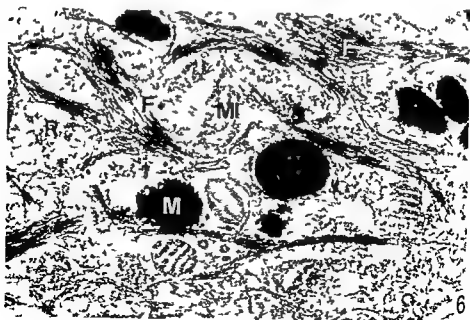
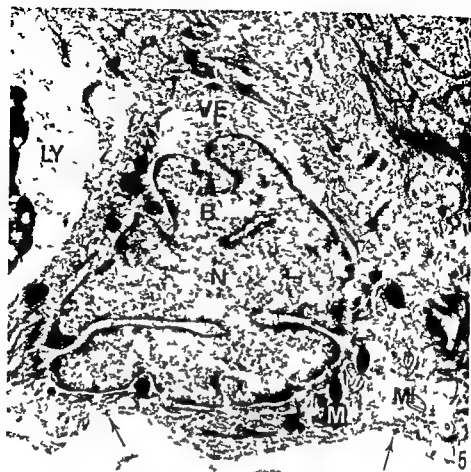
### *The basal cell*

The basal cell had a rather irregular shape (Figs 4 and 5). The nucleus was large and occupied the greatest part of the intracellular compartment. Thick bundles of parallel microfilaments, some of them surrounding the nucleus, constituted a prominent feature of the cytoplasmic content (Figs 4-6). Rather small, round mitochondria were enmeshed within the microfilament bundles (Fig 6). A Golgi complex was often seen in the supranuclear part of the cell. Free ribosomes (Fig 6) and rough surfaced endoplasmic reticulum were found in the cell periphery. A few vesicles were distributed randomly throughout the cytoplasm (Fig 5). Mature melanosomes were seen in most basal cells, located both in the perinuclear zone and in the cell periphery (Figs 5 and 6). They were not membrane bound. The cytoplasm projected numerous short and thin extensions, loosely interdigitated with those of the adjacent cells (Fig 7). In the outer part of the basal layer, adjacent cells were connected by desmosomes (not shown in the figures).

### *The melanocyte*

Melanocytes were interspersed irregularly between the basal cells. They consisted of a rounded cell body and a number of dendrite like cytoplasmic processes (Fig 7). A large nucleus was surrounded by a thin layer of cytoplasm containing numerous melanosomes. The cytoplasmic matrix was often slightly more electron lucent than that of the basal cells. Microfilaments were absent both in the cell body and the dendrites. Free ribosomes were scattered throughout the cytoplasm. Otherwise, the cell organelles were comparatively few. The cell body as well as the dendrites showed a smooth cell membrane without junctional complexes of any kind and without interdigitations with the facing cells.

The dendrites penetrated between the cells in the basal, intermediate and sometimes superficial cell layers (Figs 4 and 7). The number of melanosomes



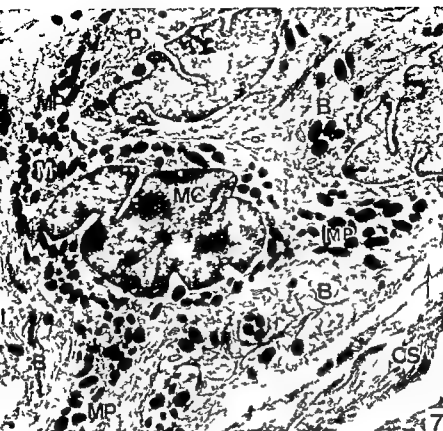


Fig. 1

cyte (MC) with dendrite like processes (MP) located between the basal (B) and polyhedral (P) cells. Melanosomes (M) are numerous in the melanocyte but they can be found in all adjacent cells. Arrow points to the basal lamina. CS conjunctival stroma. I loose interdigitations and rather wide intercellular space between two basal cells  $\times 8700$

Figs 5-6

Basal cell (B) with deeply crenated nucleus (N), bundles of microfilaments (F) and large mitochondria (MI). VE vesicle, LY lymphocyte, M melanosomes. Arrows point to the basal lamina with half desmosomes  $\times 111000$

Part of a basal cell with free ribosomes (R), melanosomes (M) and small mitochondria (MI) enmeshed within thick bundles of microfilaments (F)  $\times 33600$

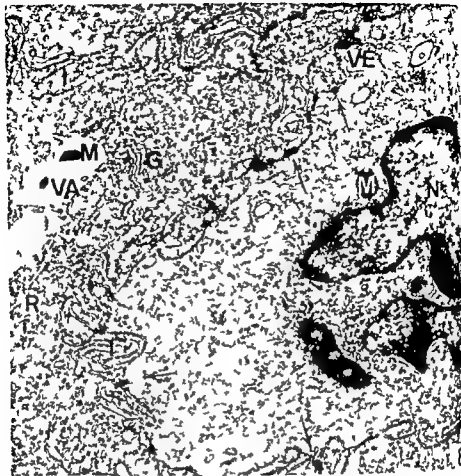


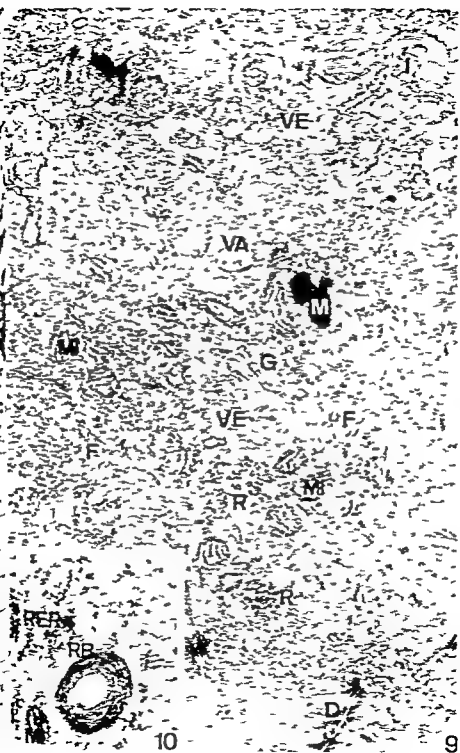
Fig 8

Two adjacent polyhedral cells (a dark one to the left and a light one to the right) in the intermediate layer. Note the difference in the electron density of the cytoplasmic matrix. The cell processes are interdigitating (I) with rather narrow and uniform intercellular spaces. Vesicles (VE) and vacuoles (VA) are located predominantly in the supranuclear part of the cells. The vesicles seem to be empty, while the vacuoles contain granulated material, membrane profiles and what appears to be melanin (M). G Golgi complex, N nucleus, R free ribosomes. Arrows point to desmosomes.  $\times 21,500$

Figs 9-10

9 The supranuclear portion of a polyhedral cell in the outer part of the intermediate layer. The cell processes show tight interdigitations (I). In certain places the cells are connected by desmosomes (D). Vesicles (VE) are concentrated in the proximity of the moderate Golgi complex (G) and in the apical part of the cell. Melanosomes appear to be lying free in the cytoplasmic matrix. F microfilaments, MI mitochondria, R free ribosomes, VA vacuole.  $\times 23,100$

10 Residual body (RB) with concentrically arranged membranes. RER short profiles of rough surfaced endoplasmic reticulum, MI mitochondrion.  $\times 33,000$



seemed to decrease toward the dendrites. Towards the end the connectives contained only a very electron-lucent cytoplasmic matrix, and it was difficult to differentiate them from other smooth, dendritic-like processes extending in horizontal planes.

### *The small lymphocytes*

The small lymphocytes found occasionally between the basal cells (Fig. 2 and 5) showed no intermediate or intermediate junctions. The nuclei of these cells were small and the cytoplasm was low in electron density with few intracellular granules. The lymphocytes extended long dendritic processes (Fig. 4, 6) in a manner similar to certain type of processes of the intermediate and superficial cells.

### *The intermediate cell layers of the peritubular zone*

In the major part of the peritubular zone the intermediate layers were composed of two distinct layers of polyhedral or wedge-shaped cells (Fig. 7). These included the basal and superficial cells. The epithelium of the tubular zone was four to five-layered. Lymphocyte cell bodies were scattered in the superficial cell layer while the processes of lymphocytes in melanocytes could be seen also in the outer layers.

### *The polyhedral cell*

The polyhedral cells were of varying sizes and shapes. They extended a large number of cytoplasmic processes, and their cell membranes were very irregular (Figs. 4, 6 and 9). In the superficial layer the circular spaces were prominent whereas in the outer layers (Figs. 8 and 9) the processes were more crowded and the cells joined by a larger number of desmosomes than in the inner layers.

The cytoplasmic matrix of most polyhedral cells in the tubular zone was electron-dense ("dark cells") (Fig. 4). However, in a moderate number of the densely stained polyhedral cells the cytoplasmic matrix was more electron-lucent, and these cells therefore appeared as "light" cells (Fig. 2). In all cases respect both cell types seemed to be identical. The nuclei of these cells were small, heterochromatic, and near the nuclear membrane (Fig. 2). A moderate number of mitochondria, some profiles of rough-surfaced endoplasmic reticulum and a rather large number of free ribosomes were scattered in the cytoplasm. Microfilaments were only rarely apparent as straight bundles. The cell bodies were grouped in loose arrangements or separated in the cytoplasm (Fig. 4).

The Golgi complex was well developed particularly in the cells of the outer layers and often located in the apical part of the cell (Fig 9) Vesicles of different sizes were seen in the vicinity of the Golgi complex Vesicles and vacuoles (the larger structures) were found in rather modest numbers in the inner cell layers whereas in the outer layers they were numerous and concentrated mainly in the apical cell compartment (Fig 9) Most vesicles had a clear appearance while many vacuoles were seen to contain finely or coarsely granulated material as well as membrane profiles (Figs 8 and 9) Melanosomes were found either free in the cytoplasm (Fig 9) or within vesicles or vacuoles as whole granules or parts of them (Fig 8) The latter arrangement was more frequently observed in the outer than in the inner cell layers

Primary lysosomes could not be positively identified What appeared to be secondary lysosomes (residual bodies) were seen in the outer layers however (Fig 10)

### Discussion

A rather detailed description of the ultrastructure of the perilimbal zone of the human conjunctival epithelium was published by Wanko et al (1964) The authors also studied the same zone in the albino rat for comparison with the human material and found no significant differences Furthermore they pointed out the resemblance between the conjunctival epithelium and stratified squamous epithelia such as the oral mucosa and the combined stratum germinativum and stratum spinosum of the skin Hogan et al (1971) emphasized the many similarities between the corneal epithelium and the limbal zone of the conjunctival epithelium of the human eye

The general ultrastructural pattern of the perilimbal zone of the guinea pig conjunctival epithelium showed good conformity with that of the human counterparts as described in the two previously cited studies However certain dissimilarities were observed and will be pointed out

The most conspicuous difference was the absence of goblet cells Sections of tissue from different meridians of the perilimbal zone of the lower half of the eye were cut, both parallel with and perpendicular to the limbus but goblet cells were not seen In human preparations Wanko et al (1964) and Radnot (1971) found an abundance of goblet cells in the perilimbal zone whereas Hogan et al (1971) described such cells in the periphery of the limbal zone According to Hessing (1968) the occurrence of goblet cells varies in different meridians and at different distances from the limbus It thus seems that differences as to location of the sections might be the main reason for the discrepancies



Another point of difference was the degree of pigmentation of the perilimbal zone. In the pigmented guinea pig the pigmentation could be seen macroscopically and light as well as electron micrographs showed the presence of melanosomes in the majority of cells in this zone. The conjunctiva of the average white human contains less melanin (Wanko et al 1964 Hara et al 1971).

Although melanin is synthesized in the melanocytes the melanosomes are observed in almost all adjacent epithelial cells which implies some kind of transport across the cell membranes. Birbeck et al (1956) suggested that the melanosomes are phagocytized by the keratinocytes of the epidermis a theory accepted by numerous investigators. Opinions differ however as to whether the keratinocyte engulfs and pinches off a portion of a melanocyte dendrite containing melanosomes (Birbeck et al 1956 Cruickshank & Harcourt 1964 Mottaz & Zelickson 1967 Prunieras 1969 Klaus 1969a Okazaki et al 1970) or takes up melanosomes extruded into the intercellular space by the melanocyte (Swift 1964). The phagocytic capability of the keratinocytes has also been demonstrated by the uptake of metabolically inert markers such as carbon particles (Platt 1963) and latex microspheres (Wolff & Konrad 1962). Since melanosomes are also found in the conjunctival epithelial cells adjacent to the melanocytes it seems reasonable to expect an identical transport mechanism, i.e. phagocytosis by the basal and polyhedral epithelial cells.

The presence of two kinds of polyhedral cells "dark" and "light" ones has not previously been reported for the conjunctival epithelium. However, an electron micrograph of intermediate layers of human conjunctival epithelium published but not commented upon by Carroll & Kuwabara (1968) showed polyhedral cells with different electron density of the cytoplasmic matrix. Light and dark cells were also observed in the similar epithelium of pterygium (van der Zyp et al 1970) and interpreted as reflecting the differences in metabolic activity of the cells. Dark and light cells were observed in various normal and pathological animal tissues by many investigators and generally interpreted as artifacts of immersion fixation (Palav et al 1962 Ganote & Moses 1968). This interpretation was supported by the simultaneous findings of changes in intracellular organelles characteristic of poor fixation. However, dark and light cells were also found in the epithelium of the choroid plexus after perfusion fixation (Dohrman 1970). It was suggested that the dark and light cells represent varying stages of cellular hydration. Our material showed no evidence of poor fixation.

The finding of small lymphocytes in the cell layers of the perilimbal region possibly including some immunologically competent cells suggests that the conjunctival epithelium is capable of immune defense.

The intercellular spaces were wider close to the basal lamina than farther out in the epithelium. This arrangement might imply that the intercellular route of transport is of significance basally in the epithelium whereas farther out the intracellular route might be the more important one.

As the cells moved from the basal layer and reached the intermediate layers the Golgi complex and the vesicles increased in size and number and were concentrated in the apical part of the cell. At this level some vacuoles were seen to contain finely or coarsely granulated material, membrane profiles and/or melanosomes. The vesicles observed in the vicinity of and most likely originating from the Golgi complex probably contain products synthesized or packaged there: mucopolysaccharides, hydrolytic enzymes etc. (Rambourg 1971, Rhodin 1974, Lloyd & Beck 1974). Primary lysosomes however cannot be identified with certainty solely on the basis of their morphology. Other vesicles could be part of a transport mechanism across the cell membrane, the smallest of them possibly being the pinocytotic vesicles. The presence of a few multivesicular and residual bodies as well as the findings of granulated material, membrane profiles and also what appeared to be partly decomposed melanosomes within many vacuoles can be interpreted as signs of autophagocytosis (Hori et al. 1968, Klaus 1969b, Lloyd & Beck 1974).

An abundance of filaments is found in cells with a need for a well developed cytoskeletal system (Fawcett 1966, Rhodin 1974, Gipson 1977). Besides there is increasing evidence that the microfilaments are the contractile machinery of a non muscle cell (Wessells et al. 1971). As such they participate in most forms of cell movement phenomena: cytoplasmic streaming, cell shape changes and locomotion (e.g. cell sliding in connexion with corneal wound healing) and phagocytosis (Wessells et al. 1971, Bretscher & Raff 1975, Burnside & Laties 1976, Gipson 1977, Gipson & Anderson 1977). Several of these qualities also seem to be relevant in connexion with the conjunctival epithelium and will be further discussed in a following paper (Latkovic & Nilsson 1979a).

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## THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

### II The Superficial Layer of the Perilimbal Zone

BY

STEFAN LATKOVIC and SVEN ERIK G NILSSON

The ultrastructure of the superficial cell layer of the perilimbal zone of the guinea pig conjunctival epithelium as seen in transmission and scanning electron micrographs is described. The major interest is focused on the apparatus of vesicles, vacuoles and cysts and their possible significance in connexion with transport functions and autophagocytic processes. The importance of the cell surface components and the role of the microfilaments in phagocytosis are also discussed.

**Key words:** conjunctival epithelium – perilimbal zone – guinea pig – ultrastructure – transmission electron microscopy – scanning electron microscopy

In the previous paper (Latkovic & Nilsson 1979a) the basal and intermediate cell layers of the perilimbal conjunctival epithelium of the guinea pig were examined. The present paper concerns the ultrastructure of the superficial cell layer of the same zone as observed in transmission and scanning electron microscopy. This series of papers, also including the other zones of the conjunctival epithelium (Latkovic 1979a,b) is intended as a basis for a study of phagocytosis in this epithelium now in progress (Latkovic & Nilsson 1979b). In this connexion the superficial cells are of particular importance.

The superficial layer of the perilimbal zone of the conjunctival epithelium has previously been studied by Wanko et al (1964), Radnot (1971a) and Pfister (1975). A similar epithelium that of the limbal zone has been described by

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Hogan et al (1971) The surface morphology of the human palpebral conjunctiva was examined by Takakusaki (1969) Dark et al (1974) and Greiner et al (1977)

## Materials and Methods

The eyes of four clinically healthy adult pigmented guinea pigs were perfused with 4% glutaraldehyde for the present study Specimens of perilimbal conjunctival epithelium were prepared for transmission electron microscopy according to the method described earlier (Latkovic & Nilsson 1979a)

For scanning electron microscopy the acetone dehydrated specimens were subjected to critical point drying using liquid carbon dioxide as the transitional fluid The specimens were mounted on specimen holders with conducting silver paint and a vacuum evaporator with carbon and gold and examined at an angle of 45° in a JEOL SM 1 scanning electron microscope

## Observations

The superficial layer of the perilimbal zone of the conjunctival epithelium of the pigmented guinea pig consists of a single layer of polyhedral cells It is continuous with the corresponding layer of the limbal and bulbar zones The cell nucleus was large and irregular (Fig 1) The cells were rich in mitochondria most of them concentrated in the supranuclear part of the cell (Fig 1) The number of free ribosomes was moderate and rough surfaced endoplasmic reticulum occurred only sparsely The microfilaments appeared to be fewer in the superficial cells than in the deeper cells and they were dispersed in the cytoplasm However a condensation of microfilaments was found subjacent to the free surface which at low magnification was seen as a discontinuous electron dense band parallel to the plasma membrane (Figs 1 and 2) The cytoplasmic matrix was of moderate electron density (Fig 1) and all cells of the superficial layer were uniform in this respect

*Fig 1*

Part of a polyhedral cell in the superficial cell layer A multitude of vesicles (VE) and vacuoles (VA) is the most prominent feature of the cytoplasm The vacuoles contain granulated material membrane profiles and melanosomes (M) in what appears to be different stages of decomposition An intracellular cyst (CY) is located near the free surface Microvilli project from all sides into the cavity The dark discontinuous band under the microvillous surface represents aggregated filaments Interdigitating processes from adjacent cells MI mitochondria MV microvilli N nucleus NO nucleolus R free ribosomes  $\times 1,500$





Vesicles and vacuoles were prominent components of the apical part of the cytoplasm (Figs 1 and 2). Most vesicles were small and empty looking, often surrounding a well developed Golgi complex or aggregated subjacent to the free surface of the cell (Fig. 2). The vacuoles were single or clustered in the latter case often communicating (Fig. 1). They contained granulated material, membrane profiles, small vesicles and melanosomes (Figs 1 and 2). Some dense structures that seem to represent residues of partly degraded melanosomes were also found in the vacuoles (Fig. 1). Multivesicular bodies were occasionally observed in the apical part of the cell (Fig. 4).

Cytoplasmic processes of adjacent cells interdigitated tightly (Fig. 1) and the intercellular space was narrow. The cell membranes were connected by desmosomes. Immediately below the free surface of the epithelium cells were bound together by tripartite junctional complexes (zonula occludens/tight junction, zonula adherens and macula adherens/desmosome) which closed off the intercellular space partly seen in Fig. 2.

A large number of microvilli projected from the free surface of all cells (Figs 1-3, 5-11). That which at low magnification appeared as a thin uneven coat on the surface of the microvilli (Fig. 3) was seen at high magnification (Fig. 11) as irregular filaments protruding from the tips and sides of the microvilli. In other respects the plasma membrane of the microvilli did not differ from that of the rest of the cell. Straight microfilaments ran axially through the centre of the microvilli and extended into the cytoplasm of the cell body (Fig. 3).

Scanning electron microscopy of the free surface of the epithelium showed that the cells generally had an irregular pentagonal or hexagonal shape (Fig. 5). The cell borders were somewhat elevated above the cell surface (Figs 5 and 6). The number and length of microvilli varied somewhat from cell to cell (Fig. 5).

#### *Figs 2-4*

2 Supranuclear part of a superficial cell. Small empty looking vesicles (V) are aggregated subjacent to the free surface and also around the Golgi complex (G). Vacuoles (VA) contain granulated material and melanosomes (M). F microfilaments. MV microvilli. N nucleus. R free ribosomes. RER rough surfaced endoplasmic reticulum. JC junctional complex.  $\times 28\,100$ .

3 Microvilli. In certain places an uneven coat (arrows) can be seen on the surface of the microvilli. Straight microfilaments (F) run axially through the microvilli. A delicate network of microfilaments can be seen subjacent to the cell membrane.  $\times 68\,500$ .

4 A multivesicular body surrounded by mitochondria.  $\times 56\,500$ .

## *Ultrastructure of Conjunctal Epithelium II*

Some of the cells demonstrated small areas often slightly elevated where the microvilli were shorter and fewer (Fig 5) or sometimes absent (Fig 6). Such an area was invariably located near the cell border usually where three cells came together (Figs 5 and 6). Certain cells showed an opening in the area

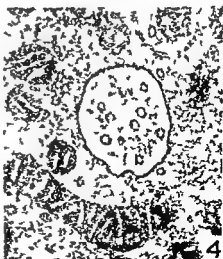




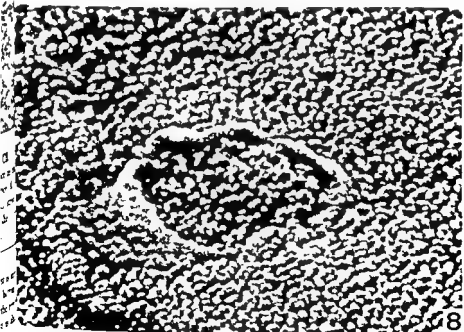
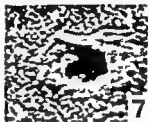
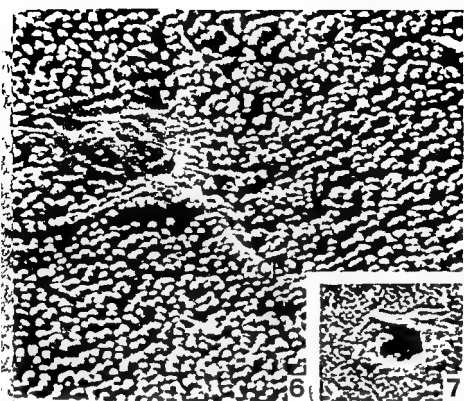
Fig 5

Scanning electron micrograph of epithelial surface. The cell borders (CB) are elevated and the cells have an irregular shape. Depending upon the variance in density and length of the microvilli the cells have a lighter or darker appearance. Arrows point to areas where the microvilli are fewer and shorter. Slightly darker stripes run diagonally across the micrograph represent shadows caused by the folds in the specimen. C.Y. unroofed intracellular cyst  $\times 6500$

Figs 6-8

Intracellular cysts in three stages. The bare elevated roof of a cyst is seen near the cell border (CB) (Fig 6). Through an opening in the roof the bottom of a cyst covered with microvilli can be observed (Fig 7). What seems to be the remains of the roof of a cyst are demonstrated in Fig 8. The bottom of the cyst is almost at the level of the cell surface.

Fig 6  $\times 25100$  Fig 7  $\times 8000$  Fig 8  $\times 26000$





ading to an intracellular cyst the bottom of which was covered with short microvilli (Fig 7) What seems to be the remnants of the membranous roof of such a cyst are demonstrated in Fig 8

In transmission electron micrographs cysts of this kind were found only in the cells of the superficial layer located in the proximity of the free surface and near the cell border (Figs 1 9 and 10) They were identified by a number of microvillous extensions from the walls of the cavity (Fig 1) In some sections an opening through the roof of the cyst was seen (Figs 9 and 10) The cavity of the cyst appeared empty in all sections where an opening to the free surface was present (Figs 9 and 10) However in sections where no opening was found granulated or strand like material and membrane profiles were often seen in the cysts (Fig 1) the same kind of material that was characteristic of the vacuoles described above In a few cases what appeared to be the remains of melanosomes were also observed in the cavity

Cells containing the cysts did not show any differences in size or intracellular content as compared to cells without cysts

## Discussion

Comparisons of the superficial epithelial cells of the guinea pig conjunctiva with the human counterparts as described by Wanko et al (1964) and Radnoti (1971a) for the perilimbal zone and by Takakusaki (1969) Dark et al (1974) and Greiner et al (1974) for the palpebral/tarsal zone showed good general conformity The dissimilarities concerned only the number and distribution of some intracellular organelles (mitochondria microfilaments vesicles vacuoles and melanosomes) and the goblet cells which were absent in the guinea pig The last two details were discussed in the preceding paper (Latkovic & Nilsson 1979a)

### *Figs 9-11*

Intracellular cyst (CY) with an opening in the thin roof Inside the cyst microvilli (MV) project only from its bottom The roof is lacking microvilli on both sides The cyst is located near the cell border with a junctional complex (JC) 1 interdigitating processes between adjacent cells  $\times 10\,300$

Shallow almost unroofed intracellular cyst (CY) near a junctional complex (JC) The bottom is almost at the level of the cell surface and covered with rather sparse short microvilli (MV)  $\times 12\,000$

High power electron micrograph showing filamentous structures protruding from the tips of microvilli (obliquely cut) of a conjunctival epithelial cell  $\times 167\,000$

The microvillous surface of the guinea pig conjunctival epithelium was seen at high magnification to be covered by filaments projecting from the membrane. Similar filaments have been observed on a number of different cells. They are composed mainly of polysaccharides and considered as an integral part of the cell membrane. These filamentous structures occur only on the free surface of a cell which can react with agents in the external environment. They play a role in recognition, contact and adhesion phenomena of the cell surface, e.g. in connexion with phagocytosis. The filaments represent a variation of the glycocalyx covering all cell surfaces. For extensive review of this voluminous subject see Martinez Palomo (1970), Winzler (1970), Lebourcq (1971) and Cook & Stoddart (1973). According to Parsons & Subje (1972) however, distinction must be made between the cells that derive a thick coat of free polysaccharides from present mucus-secreting cells and other cells of more general type with thinner coats. A coat of acid polysaccharide on the surface of the human conjunctiva was demonstrated by Radnot (1971).

The microprojections also greatly increase the actual surface of the epithelium which according to Harding et al. (1974) should augment the process of diffusion and active transport and also enhance the total activity of enzymes associated with this region of the plasma membrane (Boyd & Parsons 1964).

The apical part of the polyhedral cells in the superficial layer was remarkably rich in vesicles and vacuoles. These have been observed by a number of authors (Wanko et al. 1964, Takakusaki 1969, Hogan et al. 1971, Dark et al. 1974) but no proposals as to their function have been offered. It is suggested that the primary lysosomes originate in association with the ribosomes and the Golgi complex (Lloyd & Beck 1974). Some of the membrane-bound vesicles observed in the vicinity of the latter therefore could be primary lysosomes. It is not possible to identify lysosomes solely on the basis of morphology without a positive reaction for acid hydrolases. However, the presence of some of the secondary phagosomes (autophagic vacuoles, residual and multivesicular bodies) could serve as indirect evidence for the occurrence of lysosomes in the superficial epithelial cells.

Most vacuoles, particularly the large ones, had a content suggesting autophagy, i.e. granulated material, membrane profiles and melanosomes some of which appeared to be in different stages of degradation. Many of the intracellular cysts in sections where an opening was not observed contained material at least partly of the same kind as that of the vacuoles, whereas cysts in which an opening was demonstrated seemed to be empty. The interpretation of the whole procedure could be that the vesicles supply necessary enzymes, whereas vacuoles and intracellular cysts might be sequential steps in an autophagocytic process where cytoplasmic organelles are enclosed and directed

id which ends with the opening unroofing and emptying of a cyst. This may also be seen as an expression of ageing of the cells. A participation of the cysts in other functions cannot however be fully excluded.

Intracellular cysts (or crypts) with an opening were observed in scanning electron micrographs of corneal and conjunctival epithelium of the rabbit (Fister 1973, 1975; Schmidt-Martens et al. 1976) and of tarsal conjunctival epithelium of the human (Greiner et al. 1971). The latter authors interpreted the open crypts as collapsed and empty goblet cells. In our material this would not be the case since goblet cells were not found in the perilimbal zone. Fister suggests that the cysts initiate the desquamation of the cells, thus exposing the second cell layer, an interpretation accepted also by Schmidt-Martens et al. (1976). Our transmission electron micrographs do not support this theory. So far we have not observed any full thickness holes, the depth of the cysts representing only a fraction of the total cell thickness, and we have already shown that the microvilli are present on the walls of the cysts without exposing the second cell layer.

The increasing number of vesicles and vacuoles observed as the cells move from the basal layer towards the surface of the epithelium and their concentration in the apical part of the cells further support the presence of an active transport system directed outwards. On the other hand, a clustering of small vesicles subjacent to the free surface also suggests a participation of vesicles in a transport inwards across the plasma membrane including pinocytosis.

In the superficial epithelial cells the microfilaments appear (1) as loose microfilaments randomly distributed in the cytoplasm, (2) as a thin zone of aggregated microfilaments subjacent to the free surface of the cell, and (3) as core microfilaments running axially through the microvilli. Microfilaments are found in almost every cell and are regarded as the contractile machinery of non-muscle cells (Wessells et al. 1971). The arrangements of the microfilaments underlying the free surface of the epithelial cells of the guinea pig conjunctiva resembles that of the terminal web filaments of the intestinal epithelium where an active movement of microvilli through interaction of core filaments and terminal web filaments was demonstrated (Rodewald et al. 1976). Participation of subplasmalemmal filaments in the membrane movements in phagocytosis has also been demonstrated for macrophages (Allison et al. 1971; Reaven

Axline 1973; Axline & Reaven 1974) and for the retinal pigment epithelium (Burnside & Laties 1976). A similar aggregation of the microfilaments was observed in the superficial cells of the corneal epithelium where they participate in cell movements during wound healing (Gipson & Anderson 1977). It seems likely that the same mechanisms are also applicable to the conjunctival epithelium.



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# PULSE INDUCED INTRAOCULAR PRESSURE VARIATION AND RETROBULBAR ANAESTHESIA WITH AND WITHOUT ADRENALINE

BY

PEKKA POHJANPELTO

The intraocular pressure (IOP) and pulse induced IOP variation (IOPV) were registered before and five min after retrobulbar anaesthesia (RBA) in 34 operations. The every second operation the anaesthetic (bupivacaine or lidocaine) was supplemented with adrenaline (epinephrine). Both IOP and IOPV were significantly lowered compared with the unanaesthetised fellow eye. The change in IOP was 16.8% with adrenaline and 15.1% without it. IOPV decreased 50.6% with adrenaline and 36.9% without adrenaline. It is concluded that RBA reduces the intraocular blood supply and this is probably one reason for the IOP lowering influence of RBA. Although to a smaller degree this vascular effect is also achieved without adrenaline.

*Key words:* adrenaline - epinephrine - retrobulbar anaesthesia - ocular surgery - intraocular pressure - intraocular blood supply

Adrenaline (epinephrine) added to the anaesthetic is known to cause vasoconstriction and to decrease local blood circulation though the reaction may differ in different tissues (Dhuner & Lewis 1966). Reduction of the intraocular blood volume might be beneficial in many eye operations. It would lower intraocular pressure (IOP) and leave more space for the vitreous. Vasoconstriction in the choroid might lessen the risk of expulsive haemorrhage during the operation and the danger of haemorrhage when a perforation is performed in operations for detachment of the retina might also be smaller.

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On the other hand excessive vasoconstriction might cause detrimental ischaemia in the eye. Caution has been advised in the use of adrenaline because of the danger of gangrene from local blocks in extremities such as fingers and toes (Moore 1955). Very rarely a retro ocular injection of adrenaline may induce spasm of the central retinal artery and lead to optic nerve atrophy (Stallard 1973).

After retrobulbar injection of either lidocaine or adrenaline a 40–50% decrease in corneal indentation pulse amplitudes have been demonstrated by use of dynamic tonometry (Syrdalen & Horven 1970). The simultaneous injection of both drugs augmented the reduction significantly. The purpose of the present study was to elucidate the potential effect on intraocular blood circulation of retrobulbar anaesthesia (RBA) and adrenaline added to the anaesthetic by recording pulse induced IOP variation (IOPV) before and after RBA with Alcon Applanation Pneumatograph. The potential change in IOP was studied at the same time.

### Material and Methods

The material consisted of 26 patients whose mean age was 62.0 years, range 34 to 81. These patients underwent a total of 34 operations in 32 eyes at which the measurements were made. There were 12 iridectomies, nine cataract extractions, nine photocoagulations, one retinal detachment operation, one dissection, one cyclodialysis and one trabeculectomy. Six patients have been operated on and examined more than once. Iridectomy was performed on two patients and a cataract extraction on one patient was also performed in the fellow eye on a different occasion. Two patients underwent photocoagulation three times and one patient twice.

The anaesthetic agent was 0.5% bupivacaine (Marcain Astra) in 12 operations and 0.5% bupivacaine + adrenaline 5 µg/ml (Marcain Adrenaline Astra) in an equal number of operations. 2% lidocaine (Lidocain Orion) was the anaesthetic agent on five occasions and 2% lidocaine + adrenaline 5 µg/ml (Lidocain Adrenalin Orion) was used five times. The volume of anaesthetic injected was 2 ml.

Hyaluronidase (Hyason 150 UI/10 ml of anaesthetic) was added to the anaesthetic in nine operations. As hyaluronidase did not seem to affect the results it was disregarded in analysing the results.

The photocoagulations were performed without pre medication. The other patients were given the routine pre medication in use at our hospital: pethidine/chloride 1 mg/kg and generally atropine 1 mg/10 kg.

The anaesthetic was injected through the lower lid from the border of the lateral third over the lower rim of the orbit by directing a 30 mm long & possible needle inwards and upwards as deep as it would go 1.5 ml of anaesthetic was injected at this site The remaining 0.5 ml was injected while withdrawing the needle a few millimetres

The anaesthetic was chosen at random Adrenaline was used with the anaesthetic in every second operation and every other anaesthesia was performed without it

The unanaesthetised eye served as the control The examinations were made with an Alcon Applanation Pneumatograph that enables the recording of IOPV four times magnified IOP and IOPV were measured in both eyes immediately before anaesthesia and 5 min after it The measurement was made first on the eye to be operated on In the first eight operations the measurements were made 2.5 and 10 min after anaesthesia but as the results were similar in all these measurements only the results obtained at 5 min were taken into consideration From 1 to 2 drops of oxibuprocaine was used for surface anaesthesia

The statistical analysis was made with sign test

## Results

The mean IOP and IOPV values before RBA and five min after it are shown in Table I There was no difference in the effect on the results between bupivacaine and lidocaine and they were therefore grouped together in the statistical analysis

The high IOP values are attributable to the higher readings with the applanation pneumatonograph in the supine position than those given by Schiotz tonometer for the same eye There were also some hypertensive eyes

The fall in IOP in the anaesthetised eye is highly significant ( $P < 0.001$ ) statistically compared with the control eye which also showed a slight drop ( $P < 0.01$ ) The reason for the reduction of IOP in the control eye is not clear It might be due to a central mediated sympathetic reaction However although the pneumatonograph has not a tonographic effect (Langham 1974) the role of repeated tonometry can not be excluded The average decrease in IOP in the anaesthetised eye was  $16.8 \pm 11.2\%$  when adrenaline was added and  $15.1 \pm 9.4\%$  without it The difference between these two groups is not statistically significant

The IOPV also decreased ( $P < 0.001$ ) compared with the control eye in which it too fell slightly ( $P < 0.01$ ) An example of a high response is given

Table I

Mean values of intraocular pressure (IOP mmHg) and pulse induced intraocular pressure variation (IOPV mmHg) before and five min after retrobulbar anaesthesia (RBA)

Anaesthetic solution	No of operations	Test eye				Control eye			
		IOP		IOPV		IOP		IOPV	
		Before RBA	After RBA	Before RBA	After RBA	Before RBA	After RBA	Before RBA	After RBA
0.5% bupivacaine	10	24.8 ± 4.5	20.8 ± 3.6	2.6 ± 1.3	1.1 ± 0.5	24.5 ± 4.3	23.1 ± 3.4	2.2 ± 0.9	2.4 ± 1.0
0.5% bupivacaine + adrenaline	12	6.7 ± 3.0	2.0 ± 3.1	2.6 ± 0.9	1.0 ± 0.1	24.0 ± 1.7	23.8 ± 2.6	2.4 ± 0.7	2.2 ± 0.8
0.5% lidocaine	5	21.8 ± 2.3	19.2 ± 1.3	2.0 ± 0.9	1.1 ± 0.4	22.8 ± 3.6	20.2 ± 3.8	2.2 ± 0.1	1.9 ± 0.7
0.5% lidocaine + adrenaline	3	24.6 ± 4.7	21.0 ± 3.3	1.8 ± 0.1	0.9 ± 0.2	22.4 ± 3.0	21.2 ± 2.6	1.8 ± 0.1	1.7 ± 0.4

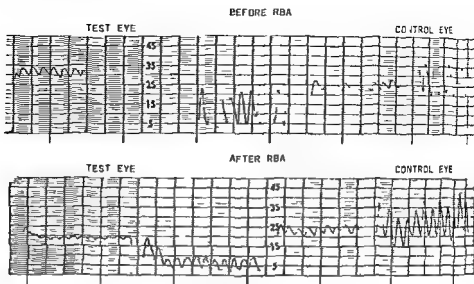


Fig 1

Records of IOP and IOPV before and five min after RBA with 0.5% Marcain Adrenalin. This 60 year old female patient had the reduction of IOP from 37 mmHg to 19 mmHg. IOPV was lowered from 40 mmHg to 19 mmHg.

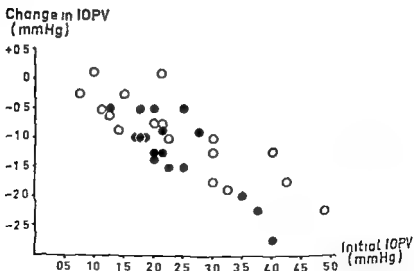


Fig 2

Change in IOPV five min after RBA (with adrenaline ● without adrenaline ○)

Table II

Mean values of pulse volume before and five min after retrobulbar anaesthesia (RBA)

Anaesthetic solution	No of operations	Pulse volume (ul) of the test eye		Pulse volume (ul) of the control eye	
		Before RBA	After RBA	Before RBA	After RBA
0.5% bupivacaine	12	34 ± 15	25 ± 09	35 ± 10	35 ± 11
0.5% bupivacaine + adrenaline	12	35 ± 09	20 ± 06	33 ± 09	32 ± 09
2% lidocaine	5	30 ± 11	18 ± 06	30 ± 09	29 ± 08
2% lidocaine + adrenaline	5	26 ± 04	13 ± 03	27 ± 08	21 ± 03

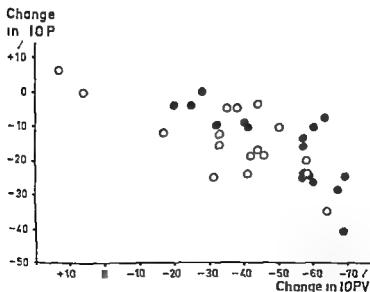


Fig 3

Effect of RBA on IOP and IOPV. The change five min after RBA has been expressed in percentages (with adrenaline ● without adrenaline ○)



sented in Fig 1 The decrease in IOPV was more evident with adrenaline than without it The average drop in IOPV when adrenaline was given was  $59 \pm 16.1\%$  and without it  $36.2 \pm 20.6\%$  (Fig 2) However the difference was not statistically significant which may be due to the smallness of the material.

In assessing the results it must be borne in mind that when IOP falls the same pulse volume causes a smaller IOPV (Bynke 1968) However the drop in IOP established in the study could not cause such a great change in IOPV in the anaesthetised eye thus it was a real decrease in pulse volume that was at issue The slight decrease in IOPV in the control eye was due to the drop in IOP and did not signify a change in pulse volume The approximate values of the pulse volumes were calculated from the mean relationship between IOP and the intraocular volume changes (Langham 1974) The mean results are presented in Table II

There was some correlation between the changes in IOP and IOPV (Fig 3) The drop in IOP was associated with a distinct decrease in IOPV The drop in IOP was  $\geq 20\%$  12 times IOPV decreased by over 50% in 10 of them A reduction of IOPV occurred in two cases and neither of them showed a decrease in IOP In some eyes however even a great change in IOPV was associated with only a slight drop in IOP

The material was divided into three groups according to the change in pressure and the average pulse volumes were calculated (Langham 1974) The results are presented in Table III The mean reduction of pulse volume was greater when IOP fell more and it seems that IOP would have declined more readily if the pulse volume was great to begin with

Table III

Intraocular pressure change and mean values of pulse volume before and five minutes after retrobulbar anaesthesia (RBA)

Group	No of operations	Original mean IOP (mmHg)	Pulse volume (ul)	
			Before RBA	After RBA
I	11	24.3	$28 \pm 0.5$	$20 \pm 0.6$
II	12	24.8	$33 \pm 1.4$	$21 \pm 0.9$
III	11	25.7	$31 \pm 1.4$	$21 \pm 1.0$

Group I decrease in IOP  $\leq 10\%$  group II 11-20% and group III  $> 20\%$

## DISCUSSION

BA had a lowering effect in this study on IOP and IOPV both in the presence of adrenaline and without it. The results conform the earlier observations of Syrdalen and Horven (1970). The lowering of IOPV was obviously attributable to the decrease in the intraocular pulse volume which probably implies that the intraocular blood supply diminished in conjunction with RBA. Lidocaine and bupivacaine did not have a decreasing effect on the circulation when injected subcutaneously or intramuscularly (Dhuner & Lewis 1966). A speculative reason for the decrease in intraocular pulse volume might be injection induced mechanical compression in the orbit of limited volume. The effect of adrenaline on the results was unexpectedly small.

The results obtained corroborate earlier observations of the lowering effect of RBA on IOP (Gifford 1949, de Roeth & Carroll 1955, Everett et al 1959, Ley et al 1962, Miklos & Halmai 1964, Syrdalen & Horven 1970). The contradiction to a recent study in which no fall was established in IOP remains unclarified (Gjottheberg & Ingemansson 1977).

The reason for the decrease in IOP in connection with RBA is not known. Aqueous outflow does not improve after RBA and it has been thought therefore that what is in question is reduced formation of aqueous due to the blocking of the ciliary ganglion (de Roeth & Carroll 1955). Relaxation of the ciliomotor muscles has been proffered as one possibility (Kettesy 1961). Atkinson (1934) believed that the fall in IOP is caused by arterial constriction when epinephrine is used. The same opinion was held by Gartner (1959) who considered that there may also be a reduction of aqueous secretion due to the constriction of the vessels supplying the ciliary body.

The results of the present work support the view that RBA lowers IOP at least partly by a direct vascular effect. It is not necessary to add adrenaline to the anaesthetic to produce this effect. The reduction in the blood supply probably causes a decrease in the quantity of intraocular blood. The free anastomosis of the venae vorticosae and other orbital veins with the facial vein would allow free exit of the blood (Atkinson 1934).

Adrenaline added to the anaesthetic agent had such a small effect on the intraocular blood supply that its use with RBA is not of great significance in this respect. However, when an eye with poor circulation needs RBA it is safer to use anaesthetic agent without adrenaline.

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## THE EFFECT OF OXAZEPAM ON OCULAR READAPTATION TIME

BY

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The readaptation time (RAT) is the interval during which a person exposed to a bright intense light flash cannot perceive a given target. In this study the target used was an optokinetic pattern and the elicited nystagmus (OKN) was registered with electrooculography (EOG) thus giving an objective registration of RAT. Oxazepam in therapeutic doses was given to five healthy subjects and the RAT and serum concentrations of the drug were registered simultaneously at different time intervals. An almost parallel increase of RAT and serum concentration of oxazepam was recorded. This suggests that RAT reflects the depressant effect on the CNS of this drug and it may be used as an objective method of following the clinical effect of a depressant drug as a function of time after intake.

**Key words:** optokinetic nystagmus (OKN) - readaptation time (RAT) - oxazepam - photo stress

Among the problems in the evaluation of the hypnotic and sedative effects of drugs are the difficulties in finding methods which are sufficiently sensitive and of obtaining objective recordings of these.

In this study registration of ocular readaptation time (RAT) following a

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brief exposure to glare is utilized. The method was used in a previous study to register effects of alcohol on healthy human subjects (Hogman et al 1976).

The aim of the present study is to determine whether effects on RAT can be recorded after intake of oxazepam in clinical doses. It also examines the feasibility of using RAT to register the effect of oxazepam on the human central nervous system (CNS) including the retina as a function of time after intake.

## Material

Five healthy male subjects aged 26–33 years volunteered for the study. None of them had consumed drugs during the previous four weeks before investigation and smoking was not allowed during the experiment.

## Methods

### Recording of readaptation time

Readaptation time (RAT) was determined by registering the pause in optokinetic nystagmus (OKN) that appears after photo stress using electro-oculography (EOG).

An optokinetic pattern is presented covering 25% of the subject's field of vision. The glaring light source covers a somewhat larger area. The luminances of the background and of the glaring light are constant ( $10^{-4}$  cdm<sup>2</sup> and  $10^4$  cdm<sup>2</sup> respectively). The method has been presented elsewhere (Tengroth et al 1976). First an initial recording was conducted on every subject at 8 a.m. to establish individual reference values for the subsequent registrations after drug intake. Thus, after intake of 20 mg oxazepam (Serepax®) per os, RAT was measured after 15 and 45 min. Thereafter, once every 30 min up to 300 min and then once every 60 min up to 405 min. Venous blood samples were drawn 30, 45, 75, 195, 315 and 435 min after intake.

### Statistical analysis

Each RAT value used in the statistical analysis was the median of five separate measurements made at intervals of 1 min. RAT changes at different points of time were tested for significance in a one-way repeated measures analysis of variance design. A trend analysis using orthogonal polynomials was also performed (Winer 1971). In order to obtain an equal interval time scale, the RAT value registered 30 min after intake was excluded from the analysis. A *P* value less than 0.05 was considered biologically significant in this study.

# termination of oxazepam in plasma

Equalized plasma was extracted with benzene (containing 1.5% v/v isoamylalcohol). After centrifuging an aliquot (2-3 µl) of the clear supernatant was analyzed by gas-liquid chromatography using a Varian Aerograph 1400 equipped with a <sup>63</sup>Ni detector. Internal standard 2-amino-5-chlorobenzophenone (ACB) was used. The chromatographic column was 6 ft glass packed with 1.5% OV 17 on Chromobond WHP (80-100 mesh). Carrier gas was nitrogen, ultra pure gas flow 30 ml/min. Temperature of the injector was 290°C, of the column 210°C and of the detector 300°C. Under these conditions the retention times were 2.3 min for ACB and 5.6 min for oxazepam. The calculations of the oxazepam contents of the samples were performed by a standard graph in which the peak height ratios  $\frac{\text{oxazepam}}{\text{ACB}}$  were plotted against known concentrations of oxazepam.

## Results

The changes in RAT at different points of time after intake of 20 mg oxazepam (Serepax®) are shown in Fig. 1 and the results of the statistical analysis in Table I. There was a statistically significant change of RAT mainly due to the non-linear components of the trend over time. Maximal RAT prolongation of the group was recorded after 2 h and the base line was reached approximately 5 h after intake. A considerable inter-individual variation of the maximal prolongation of RAT was observed - from 13 min to 165 min after

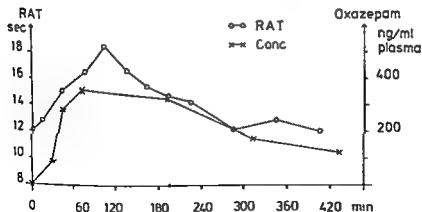


Fig. 1

Readaptation time and plasma concentration of oxazepam after intake of 20 mg Serepax®. Mean values of five healthy volunteers.

Table 1

One way repeated measures polynomial analysis of variance of RAT at various times after intake of 20 mg oxazepam (Serepar®) per os

Source	df	MS	F
T (times)	7	94.23	3.6*
T x Subjects	28	6.70	
T (linear)	1	37.72	3.6*
Error	4	10.33	
T (quadratic)	1	50.04	14.83
Error	4	3.36	
T (cubic)	1	56.59	9.53*
Error	4	5.99	

\*  $P < 0.05$  \*\*  $P < 0.01$

T = points of time after intake df = degrees of freedom MS = mean square F = F test

intake. Changes of drug concentrations in the blood also exhibited considerable inter individual variation with maximal values noted from 45 min to 190 min after intake (Fig. 2). The changes in RAT followed the changes of oxazepam concentration in the blood during the ascending phase. Thus in subject 1 where the oxazepam concentration rose slowly the maximal change in RAT was recorded later than in subject 3 who had a faster rate of absorption.

## Discussion

The effect on RAT is in agreement with the effect on critical flicker fusion (CFF) which also has been shown to be sensitive to 20 mg oxazepam per os given to healthy volunteers (Molander & Duvhok 1976). The maximal effect on CFF was shown after 3 h and the effect lasted for about 5 h. In the present study the maximal effect was recorded after 2 h and the effect lasted for 3 h.

In a previous study RAT prolongation was recorded after alcohol intake and was shown to be prolonged during the acute intoxication phase as well as during the hangover phase (Hogman et al. 1977). The observation that RAT was prolonged after intake of oxazepam in the present experiment supports the assumption that RAT reflects effects on the CNS. Furthermore the experiment shows that it is possible to register the effects of CNS depressants in the

# The Effect of Oxazepam on Ocular PAT

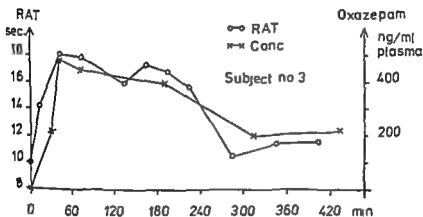
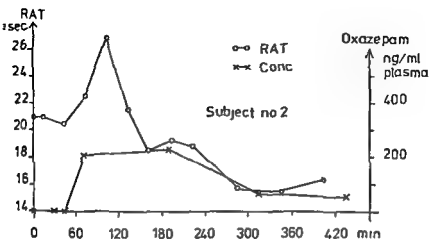


Fig 2

Readaptation time and plasma concentration of oxazepam after intake of 30 mg Serepax® in two of the volunteers

autic blood concentrations. The present study also indicates that RAT might be used to follow clinical manifestations of CNS depressant drugs as a function of time after intake.

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## A SIMPLE ROUTINE FOR OPTIC DISC PHOTOGRAPHY THROUGH A NATURAL PUPIL

### Short Communication

BY

BO BENGTSSON and C E T KRAKAU

A simple device is described which made it possible to obtain usable fundus pictures in ca 95 per cent of 2000 eyes without dilatation of the pupil. The camera is set to the patient's refraction by means of a scale on the camera extension.

*Key words:* fundus photography - Zeiss Fundus Camera - natural pupil

The examinations included in a population survey must neither be very time consuming nor disagreeable to the patients. Participants must not be frightened from coming back, if necessary, nor must they warn fellow patients not to join the survey.

In a population study at Dalby on the secondary prevention of glaucoma it was found highly desirable to document the ophthalmoscopic findings by fundus photography. In a previous survey however the discomfort caused by mydriatic drops resulted in numerous complaints.

For this reason we found it worth while to try a procedure not totally *lege artis* - namely to photograph without artificial dilatation of the pupil. Our aim was merely to produce a picture sufficiently sharp to allow us to estimate the general shape of the papilla and its degree of excavation.

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## Method

### 1 Focusing

Since the camera extension is linearly related to the patient's principal refraction a focusing scale can easily be constructed and attached to the camera (Fig 1) (Bengtsson & Krakau 1977). A refraction value is obtained by testing by Donders' method and the camera is set at this value before exposure.

### 2 Fixation

The subject is asked to fix a dim light situated about 15 degrees temporal to the second eye.

### 3 Adjustment of the camera position

The light from the flash tube of the Zeiss camera is conveyed into the examination eye by a mirror behind the front lens. Rays from the fundus reach the photographic film through a central hole in this mirror. When the camera is in the proper position the hole of the mirror is imaged by the front lens on the surface of the cornea. Correct if the hole is illuminated from behind and its image brought to sharpness on the corneal surface, we have obtained the correct distance between the camera and the examination eye.

The determination of this position is greatly facilitated by the following simple device. A tube containing a lens and two small electric bulbs is mounted on the eyepiece of the camera (Fig 2). The bulbs are placed in the focal plane of the lens and as far apart as possible in order still permit their beams to pass backwards through the camera. As a consequence, two widely separated points

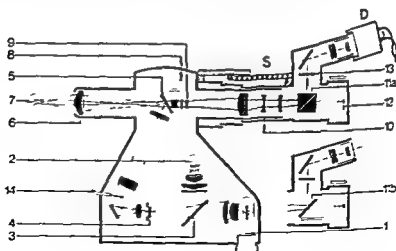
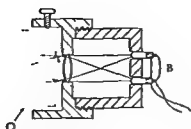


Fig 1

Diagram of Zeiss fundus camera, with scale (S) and adjustment device (D) attached.



*Fig 2*

Adjustment device mounted on camera ocular (O) B Filament bulbs

Light emerge from the front lens intersect forming a single sharply demarcated spot of light on the corneal surface of the examined eye and enter the pupil – again somewhat separated – provided that the camera is properly aligned. Even a slight error in adjusting the distance from the examined eye to the camera is revealed by a doubling of the light spot on the corneal surface. If the camera is displaced in the frontal plane the iris becomes brightly illuminated.

#### Exposure

The spot lights used for alignment of the camera are extinguished and after a short delay the shutter is tripped. Thus the pupil is allowed to dilate during a dark period preceding the flash. The retina surrounding the disc has to be kept under exposed in order to avoid over exposure of the disc. The weakest flash (60 Ws) is sufficient.

When the camera is used in this way the original filament bulb for fundus illumination has to be removed and the optic disc is photographed blindly in the sense that the eye ground is not observed at all during the whole operation.

### Results and Discussion

By this method the camera is carefully adjusted and the exposure made in less than 30 sec. This is true even in the presence of moderate ocular opacities which often cause a tiresome prolongation of the conventional procedure in elderly persons.

Our aim is merely to obtain a documentation of the papillary structures superior to drawings and verbal descriptions. This modest requirement is fulfilled in a surprisingly high percentage – no less than 95 per cent of all pictures – about 2000 – allowing reliable measurements of cup and disc sizes even in elderly persons.

In our opinion the simplicity and dependability of the method renders it well suited for recording of ophthalmoscopic findings at repeated eye examinations. Since the pupil is not dilated but somewhat constricted by the mydriatic light at adjustment the scope within which the imaging rays can be decentered is small. The parallactic variation between different pictures from this camera is therefore limited. Similarly the effects of imaging the disc peripherally are largely avoided when the subject fixes his gaze correctly.

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## JUDICIA DE NOVIS LIBRIS

*Beck Knud & Jensen Ole Aksel* External Ocular Tumours A Clinicopathologic Study of 300 Cases Textbook and Atlas Georg Thieme Publishers Stuttgart and W B Saunders Philadelphia London, Toronto 1978 64 pages 116 figures Price DM 30.-

The book is a real ophthalmological gem for the ophthalmologist who is not especially experienced in the field of tumours. It describes in an easily read and concise text with abundant illustrations the many different types of tumour of the outer eye. The illustrations are of an exceptionally high standard and comprise more than half of the book. Forty seven out of the 300 patients examined are reproduced in a most didactic fashion, whereby each individual case is demonstrated by means of a clinical, a macrohistological and a microhistological photograph.

The short text covers the main clinical and histopathological features in the course of 11 and three pages respectively. 236 out of 300 patients had skin tumours while the remaining cases were equally divided between palpebral conjunctiva, bulbar conjunctiva and caruncle.

As the tentative diagnosis were correct in only two thirds of the cases the authors recommend that any tumour or tumour like lesion be examined histologically. Clear and simple instructions are given as to when a marginal biopsy should be made and when primary total excision is preferable.

The book can be highly recommended to any clinician requiring easy, concise and clear information about the common tumours of the outer eye.

*E Gregersen*

*Handbook of Sensory Physiology* Editorial Board H Autrum R Jung W R Loewenstein D M MacKay and H L Teuber Vol. 1, part 2 The Visual System in Vertebrates Editor F Crescitelli Dept of Biology University of California Los Angeles Springer Verlag Berlin Heidelberg New York 1977 pp 813 Figs 234 Tables 23 Cloth DM 340 US\$ 149.60 Subscription price Cloth DM 219 US\$ 119.70

This is the fifth part of volume seven of this comprehensive handbook. Volume seven is dealing with all aspects of the physiology of the visual system. In 11 chapters each written by an expert in his field the present part describes the vertebrate eye through the evolution starting with the cyclosum retina and ending with the topography of vision in mammals of contrasting life style. Except for mammals the reader is not informed much about the visual pathways. The vertebrate eye and its physiology had therefore been a more appropriate title.

The first chapter on the history of vertebrates is very valuable for the non-palaeontologist pointing out the many unsolved questions in the evolution of vertebrates. Two chapters concern the fish eye and its adaptations to deep sea and photic environments and two chapters the amphibian eye. Through the eyes of geckos and turtles and their visual world the reader is conducted to the fascinating avian eye in so many respects more perfect than the human. The book shows how justified it is to consider

### *The Jules François Prize*

This prize will be awarded every three years to a research worker who has made an important contribution to ophthalmology. The candidates must be not older than 40 years of age on the first January 1980. The amount awarded will be 100 000 BF.

The candidature must be sent to the secretary of the Jules François Foundation by the candidate himself or a third person accompanied by all the necessary documents for justification. Each candidature must be sent in before the first January 1980.

Secretary J D Haenens MD II Beernaertstraat 34 8400 Oostende Belgium

### *3 Glaukom Symposium DDR*

Wird in Neubrandenburg (zwischen Rostock und Schwerin) gehalten vom 9. bis 13. 1979. Hauptthema: Das Sekundäre Glaukom.

Interessenten werden sich an Prof. G. Pietruschka, DDR-23 Rostock, Doberaner Str. 140, wenden.

### *The European Ophthalmic Pathology Society*

held its Annual Meeting in Belgrade, Yugoslavia, June 14-17, 1978. Dr. A. P. F. (Richmond, Virginia, USA) was the guest of honour. The scientific program included 39 case presentations by members and guests and 17 nations were represented. For each presentation a protocol, histopathological sections and appropriate clinical macroscopic transparencies were provided.

The majority of case presentations dealt with benign or malignant neoplastic processes involving the globe, orbit or eyelid and the discussion was concerned predominantly with classification, prognosis and management. Congenital malformations were also a prominent feature among the presentations and the remainder of the cases provided examples of systematic disorders involving ocular or adnexal tissues.

Dr. P. Dhermy (France) was elected President and Dr. W. N. Lee (UK) was elected Corresponding Secretary of the Society.

### *International Society for Clinical Electrophysiology of Vision*

The International Society for Clinical Electrophysiology of Vision will hold its 1st Symposium in Schloss Reinhardsbrunn, Friedrichroda, German Democratic Republic, 5-10 June 1979.

Two topics have been selected: Visual electrodiagnosis in systemic diseases and Visual electrophysiology and localised retinal stimulation. The official language of the Symposium is English.

For further information and registration write to:

Professor Dr. E. Schmoger, Augenklinik der Medizinischen Akademie Nordhausen, 74 50 Erfurt, GDR.

*From the Department of Ophthalmology (Head Salme Vannas)  
University of Helsinki*

# A SIMPLE METHOD FOR SCREENING OF CHILDREN WITH STRABISMUS ANISOMETROPIA OR AMETROPIA BY SIMULTANEOUS PHOTOGRAPHY OF THE CORNEAL AND THE FUNDUS REFLEXES

BY

KARI KAAKINEN

A simple screening method for detecting strabismus anisometropia and ametropia in young children by simultaneous photography of the corneal and fundus reflexes with a conventional camera and flashlight is presented. The method is the photographic application of the von Bruckner Durchleuchtung test and static skiascopy. The objective document from the external part of the eyes and the face is obtained at the same time. Model photographs with certain gaze deviation angles are presented with the method.

**Key words:** strabismus – von Bruckner Durchleuchtung test – static skiascopy – fundus reflex – corneal reflex – photography screening of vision defects and eye disorders in young children – flashlight – flickering fixation light (LED) – simple documentation

■ important for the prevention of amblyopia to make a strabismus diagnosis as early as possible in childhood (for example Pigassou (1977)). Ingram (1977) pondered the problem of screening children for visual defects. In his opinion the methods used today are still time consuming and expensive. Especially straight eyed amblyopes with anisometric refractive errors require determination of refraction in early childhood. Unfortunately examinations at this age can often be very difficult even impossible because of fear or some other lack of co operation on the part of the child. Even the cover test can be very difficult to perform when the subject is under three

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years. There is no simple and quick screening method to detect this kind of visual defect.

Von Bruckner (1962, 1965) presented the *Durchleuchtung* test in which corneal reflexes according to the Hirschberg test and the fundus reflexes and pupillary reactions are also examined with an ophthalmoscope from a distance of one metre. The strabismus or anisometropia can be diagnosed from asymmetry of these reflexes without touching the child. It seemed possible therefore to make the documented von Bruckner *Durchleuchtung* test by using a conventional camera with a flashlight instead of an ophthalmoscope. That is required is for the child to look at the camera for the brief moment when the corneal and fundus reflexes are photographed. The photographs can be later analysed by an ophthalmologist for corneal or fundus reflex asymmetry indicative of strabismus or anisometropia. In addition the fundus reflex photographs could provide information on possible changes in transparent ocular media. The approximate refractive errors too can be estimated from the different appearance of the fundus reflexes (Rosengren 1937, 1948) according to the static skiascopy.

Maddox (1902) used photography to examine strabismus angles. Later many others: Graham & Naylor (1957), Weekers et al. (1963), Joachim & G3 (1964), Breitenmoser & Wurth (1970) and Jones & Eskridge (1970) photographed the Hirschberg test and determined the stage of the deviation of the eyes from the localisation of the corneal reflexes.

Transparent ocular media are also examined quite frequently by photography generally using the Douvas & Allen (1950) principle. This has been applied in practice by Fincham (1955) and many others.

Howland & Howland (1974) presented an apparatus for photorefractometry of both eyes with a camera, but I have not found any report in the literature of the use of the combined corneal and fundus reflex photography method for both eyes for diagnosing strabismus and refractive errors simultaneously.

To discover if this was possible I constructed a simple photographic equipment for simultaneous documentation of the corneal and fundus reflexes and to test its practicality in diagnosing strabismus and refractive errors.

The first part of this investigation presents primarily the method and the experience gained with its use.

## Methods and Material

In principle it is possible by using a coaxial flashlight attached to the objective of the camera to photograph simultaneously the corneal and fundus reflex when the patient fixes his gaze on the camera objective.



*Fig 1*

The pocket flashlight Sunpak Gx 17 in front of the Canon objective FD 100 mm 1:2.8 S.C. The red flickering fixation light (LED) is in the middle under the flash unit. On the right is the oscillation circuit box of the flicker system with a switch on coupler. This was usually mounted in the back of the camera body.

A standard 35 mm single lens reflex camera (Canon EF) with an FD 100 mm 1:2.8 S.C. Canon lens was used. A weak telephoto lens was chosen because it is quite small and handy but still gives enough enlargement from a distance of one metre to detect even slight deviations of the eyes.

A Sunpak Gx 17 electronic flash unit was attached in front of the objective in the middle horizontally with the help of the Canon lens hood BT 53 so that the distance between the low edge of the flash unit and the lowest outside margin of the lens hood was 42 mm. A piece corresponding to the size of the flash unit was cut from the lens hood so that the flash unit was firmly attached by the hood.

A commercial red round flickering LED 9.5 mm in diameter was attached as a fixation light to the middle of the anterior low edge of the flash unit.

The flickering mechanism was accomplished with an oscillation circuit. The light was connected to a switch which was placed at the back of the camera body for easy handling (Fig 1).

The film material was commercial colour films. The aperture range was adjusted on the Sunpak Gx 17 flash unit's guide number chart for a photography distance of one metre which was selected as the practical distance for photography. This is the same distance that is generally used for the von Bruckner Durchleuchtung test.

**The photography technique and standardisation**

To prevent inadvertent movement of the distance scale adjustment of the objective it was fixed with a piece of adhesive tape at exactly one metre. Photography was done by hand without a stand. For focusing the camera was slowly moved to and fro until the object in the middle of the viewer was sharpest. In repeated experimental measurements focusing in this way at one metre with the camera's range finder the greatest possible error was  $\pm 1$  cm.

The photography was performed in dim light for reliable focusing. Pupils of the subjects were then larger than in bright light and the flicker-fixation light interested the children and tended to make them stare at it.

Mydriatic drops were not used because the aim was to photograph physiological situation. With young children in particular it was found useful to focus first and then switch on the flicker light just before taking the photograph asking at the same time what was flickering. This gave both a visual and auditory stimulus to the child to fix his gaze on the light.

### Telling the method

1 *Summary measurement of refraction by photography* The usefulness of the method was examined by photographing a Carl Zeiss Jena Optical Demonstration Eye with different refraction errors which were controlled with a streak retinoscope (Fig. 2).

2 *Detecting strabismus* To establish the smallest deviation angle of the eye that causes the change in intensity or colour of the fundus reflex or that can be seen as shifting of the corneal reflexes in the photographs an experimental test photo series was made. An emmetropic straight-eyed test subject was photographed when her gaze deviated from the fixation light of the camera by a certain number of degrees ( $1^{\circ}$ – $13^{\circ}$ ). The deviation angles were calculated for a slightly coarse cyclopean eye schema by using trigonometrical tangent functions in a right-angled triangle where both the catheti were known. The greater was the photography distance of one metre and the smaller the measured distance that the test subject was asked to look to the side of the fixation light of the camera.

By calculating trigonometrically it is possible to determine by using the kind of cyclopean eye schema instead of the two-eyed natural situation the error in  $3^{\circ}$  gaze deviation is  $\pm 2$  min of arc and in the case of  $13^{\circ} \pm 6$  min of arc. This is accurate enough and the cyclopean eye schema can be used because it is easy and practical for making measurements (Fig. 3).



Fig. 2

Refractive error models photographed from the Carl Zeiss Jena Demonstration Eye  
(-5.0 -4.0 -3.0 -2.0 -1.0 0 +1.0 +2.0 +3.0 +4.0 and +5.0)

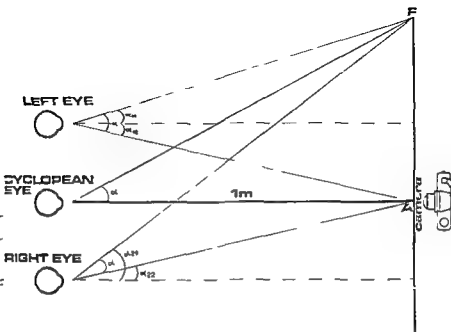


Fig 3

The gaze deviation angle of the cyclopean eye is denoted  $\alpha$  and  $\alpha_1$  for the left eye and  $\alpha_2$  for the right eye in the corresponding natural situation

$$\alpha_1 = \alpha_{11} + \alpha_1$$

$$\alpha_2 = \alpha_1 - \alpha_{22}$$

$$[\alpha - \alpha_1] = [\alpha - (\alpha_{11} + \alpha_1)] \leq 0.6 \text{ when } 3^\circ \leq \alpha \leq 13^\circ$$

$$[\alpha - \alpha_2] = [\alpha - (\alpha_1 - \alpha_{22})] \leq 0.6 \text{ when } 3^\circ \leq \alpha \leq 13^\circ$$

The photographs were taken twice and found identical. When the gaze deviated one eye presented exotropia and the other esotropia. The photographs were compared with an orthophoric photograph where the same test subject was looking straight ahead (Fig 4).

To make the examination easier, schemas corresponding to a normal strabismic situation were made by cutting the photographs in the middle and combining the gaze deviations of different degrees with half face photos in which the test subject was looking straight ahead. This gave artificial photos in which one eye was straight and the other deviating a certain number of degrees (Figs 5 and 6).

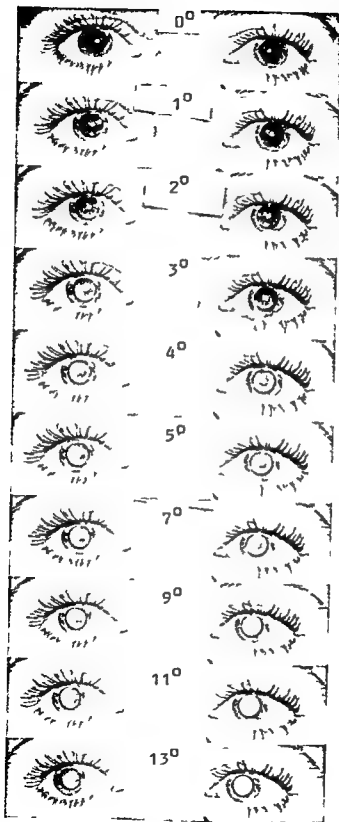


Fig. 4  
 Photographed cor-  
 and fundus reflex  
 when the gaze is  
 deviated to the right  
 1°-13°. Top the gaze  
 is fixed on the camera  
 (0°)



*Fig 5*

Artificial left esotropia 3°. Combination of the two photographs. The right eye is fixed on the camera and the left 3° to the right of the camera. The positions of the corneal reflexes are slightly asymmetrical. The different brightness of the fundus reflexes is quite apparent.



*Fig 6*

Artificial right exotropia 3°. Combination of two photographs as in Fig 5. Moderate asymmetrical corneal and fundus reflexes.

## Results

The resolution and quality of the photographs was good. In two sessions photographing the Carl Zeiss Demonstration Eye in different positions the results were identical.

In hyperopia a light crescent was found in the low part of the fundus reflex. In myopia in the upper part of the fundus reflex. The myopic crescents increased in size with the refractive error. The change could not be found when the small pupil of the eye was used. The crescent appeared in myopia around -3 D and disappeared at 1 diopter.

In the test photos where the gaze of the test subject was 15 degrees off the fixation light of the camera, the angle of just 3° convergent or divergent strabismus was detectable. The deviation of corneal reflexes while at a somewhat doubtful. It was found that deviation of



Fig 7

A 29 year old woman with anisometropia and left amblyopia. Straight eyes in cover test. Refraction in the cycloplegia in the right eye  $-3.5$  comb cyl  $+0.0$  ax  $90^\circ$  and in the left  $+5.0$  comb cyl  $+1.0$  ax  $130^\circ$ . The vision of the right eye with correction 16 and of the left 0.15. Anatomically normal eyes. There are symmetrical corneal reflexes and the hyperopic crescent is visible in the left fundus reflex.

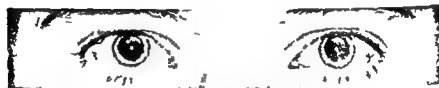


Fig 8

A 15 year old girl with  $-6.0$  myopia in cycloplegia in both eyes. Straight eyes in cover test and anatomically normal eyes. Symmetrical myopic crescents are visible in both fundus reflexes and the positions of the corneal reflexes are also symmetrical.



Fig 9

A one year old boy with an anomaly of the right upper lid retraction and small intermittent hypotropia of the right eye. Anatomically normal eyes and refraction in both eyes in cycloplegia. The cover test reveals very small right hypotropia and sometimes no movement at all. The right hypotropia and lid retraction are very apparent in the photograph. The brightness of the fundus reflexes are symmetrical.



*Fig 10*

10 year old boy with a very small left esotropia and amblyopia. Refraction in the right eye: +2.0 comb cyl +0.5 ax 90 and on the left +2.5. Anatomically normal eyes.  $V.O. dx = 13$  and  $V.O. sin = 0.1$  with the correction. In the cover test very small left esotropia in which the angle of the deviation with synoptophore is  $+9^\circ$ . A moderate asymmetry of the corneal reflexes is apparent in the photograph and the fundus reflex of the deviating left eye is lighter than in the right one.

stable while 2 changes were demonstrable but somewhat questionable. To determine the asymmetry of the corneal reflexes a normal millimetre scale was used at first to measure the distances of the corneal reflexes from theimbus. But this was not practical because even the slightest asymmetry to be measured was at least as clear by visual examination.

The colour of the fundus reflex became moderately lighter when the eye of the test subject deviated from the straight position 1 medially or laterally. When the fundus reflex kept roughly as bright except at  $+11^\circ$ – $13^\circ$  esodeviation when it became very light coloured.

In test photography sessions one year old children co-operated by looking at the fixation light. Some illustrative strabismus and a straight eyed refractive error cases of different ages are presented in Figs 7–10.

## Discussion

A one year old child can be persuaded to co-operate in dim light by looking at a flickering fixation light. Screening at that age could be possible with the method which because of its simplicity can even be set up by a technician.

Graham & Naylor (1951) found that their eye deviation measurements made by photography differed from the synoptophoric results. Joachim & Gilson (1964) reported that when they were examining by photography the measurements by synoptophore gave overvaluation in convergent strabismus and under valuation in divergent strabismus. These and the studies of Breitenmoser & Vurth (1970) and Jones & Eskridge (1970) give the impression that the deviation angle of the eye at the moment of photography can be examined with a



great degree of accuracy from the photograph which at the same time is a permanent document for future reference

The different size of the kappa angles in the right and left eye of the same person could cause an error in estimating the possibility of strabismus measuring eye deviations by comparing localisation of the corneal reflexes of the contralateral eyes. But this source of error has been shown to be of no importance in practice because photography has revealed that the kappa angles are identical in both eyes of the same person (Weekers et al. 1963)

In principle the method described here is about as accurate for measuring eye deviations as the methods cited in the foregoing. The procedure and apparatus are similar but simpler. Complicated stands that could frighten a child are not needed. The small and handy flickering LFD light has shown itself to be a practical way of attracting the attention of small children.

The method makes it possible to photograph with the conventional camera corneal and fundus reflexes simultaneously. A mere  $1^\circ$  convergent or divergent deviation of the eyes in experiments made the fundus reflexes moderately lighter than in the straight position. This concurs with the claim of von Brückner (1962) that a deviation of  $1\frac{1}{2}^\circ$  causes a change in pupillary brightness in the Durchsichtung test. It seems that this could facilitate the diagnosis of small angled strabismus cases. According to von Brückner (1962) the dark fundus reflex of the straight eye is reflected from the macula compared with the oblique parts of the retina which are lighter reflecting. The very light fundus reflex that appeared in  $+11$ – $-13^\circ$  esodeviation is apparently caused by the brightness of the optic disc.

It is clear that the method measures the refraction rather roughly according to the principle of static skiascopy in the line through the camera lens and the light source accordingly in the vertical meridian. Its accuracy is not sufficient for exact refraction measurements and the method is meant mostly for screening not only to detect strabismus but also straight eyed cases with great astigmatic or ametropic refractive errors. To obtain more accuracy for examining refractive errors especially hyperopia cycloplegic medication should be used.

The method has already been applied to a clinical strabismus and refraction material that is to be published later.

## Acknowledgments

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# FUNDUS FLUORESCEIN ANGIOGRAPHY IN FUNDUS FLAVIMACULATUS AND STARGARDT'S DISEASE

BY

NILS ANMARKRUD

Three siblings who had fundus flavimaculatus and two patients who had Stargardt's disease were studied by means of fundus fluorescein angiography. The angiograms revealed in all cases an abolished visibility of the chorioidal circulation. New flecks are usually non fluorescent. Later on hyperfluorescent areas are seen at identical places both in the pre-retinal and retinal phases strongly indicating a window effect of the retinal layer. The missing chorioidal flush is probably due to a blocking effect of the emitting and exciting light. Some of the retinal flecks may fade away leaving corresponding areas of hyperfluorescence that usually persist. In some cases however a previous fluorescent area may become non fluorescent. The similar angiographic picture may indicate that fundus flavimaculatus and Stargardt's disease are different expressions of the same disease.

*Key words:* fundus flavimaculatus - Stargardt's disease - fundus fluorescein angiography - chorioidal circulation

Stargardt described in 1909 a bilateral hereditary macular degeneration occurring in young people. In addition to an atrophic macular lesion peripheral yellow white flecks were also noticed.

Franceschetti (1963) introduced the term fundus flavimaculatus to separate a special form of tapetoretinal degeneration. In these cases the fundus was characterized by yellow white or yellow flecks at the level of the retinal pigment epithelium in the posterior pole. In 50% of the patients Franceschetti noticed an atrophic appearing macular degeneration. Others have found

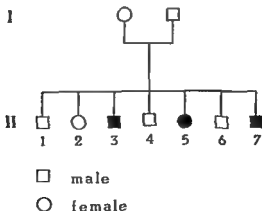
lar degeneration in 67 % (Klien & Krill 1964) In these cases the distinction between fundus flavimaculatus and Stargardts disease is difficult if at all possible In the last years the view has been put forward that these two retinal degenerations are in fact different manifestations of the same disease This is now commonly accepted (Deutman 1971 Irvine & Wergeland 1972 Franke et al 1975 Hadden & Gass 1976)

Fluorescein angiographic studies in fundus flavimaculatus were first mentioned by Ernest & Krill (1966) They concluded that the flecks and drusen were similar in location involving the retinal pigment epithelium

We have examined a family in which some of the members had reduced vision due to fundus flavimaculatus In addition we have examined two patients with Stargardts disease The purpose of this study is to describe the angiographic pattern seen in these two diseases If Stargardts disease and fundus flavimaculatus are different expressions of the same disease it would be of interest to study whether there exists a similarity in the angiographic picture

### Material and Methods

The clinical material consists of 7 siblings (Fig 1) fair haired norwegian couple Their parents were healthy with no eye symptoms and no consanguinity Three of the siblings suffered from reduced vision due to fundus flavimaculatus In addition we have examined two patients with Stargardts disease



*Fig 1*

Pedigree of family with fundus flavimaculatus Black symbols indicate affected members

A complete ophthalmological examination was performed in every patient including fundus examination by biomicroscopy Goldman perimetry E during scotopic conditions dark adaptation Ishihara colour screening fluorescein fundus angiography The angiograms were performed with a fundus camera The film used was Kodak Plus X pan After injection of sodium fluorescein solution (100 mg/ml) into an antecubital vein the angiograms were taken automatically every second during the transit of dye at then 2 5 and 10 min after injection We used Zeiss interference filter KP as excitation filter and Kodak Wratten No 15 as barrier filter

## Case Reports

*Case 1 (II 5)* A 29 year old man developed bilateral decreased central vision at age of 22 years In 1973 visual acuity was 6/20 in right eye 6/10 in the left eye ERG and dark adaptation were normal He identified only the first of the Ishihara plates and a central scotoma was present in each eye Ophthalmoscopy revealed normal optic disc and normal retinal vessels The macula in each eye showed a pericentral atrophic lesion In the posterior pole many retinal yellow white flecks of various shapes and sizes were seen (Fig 2 A) Angiograms showed numerous hyperfluorescent areas both in preretinal and arteriovenous phases with abolished visibility of the chorioidal circulation between these areas (Fig 2 B and Fig 2 C)

In 1976 the visual acuity had deteriorated to c f 5 m in right eye c f 3 m in left eye The atrophic macular lesions had increased in size but the yellow white flecks were of about the same number Some of the flecks had faded away while new ones had been added (Fig 3 A) The angiogram showed increased number of hyperfluorescent areas Some of the earlier yellow white flecks with no hyperfluorescence had faded away and corresponding to these flecks the angiogram now revealed hyperfluorescence (Fig 3 B)

*Case 2 (II 5)* A 25 year old woman sister of case 1 developed decreased central vision in both eyes at the age of 14 years Visual acuity was c f 5 m in each eye ERG and dark adaptation were normal She identified only the first of the Ishihara plates A central scotoma was found in each eye Ophthalmoscopy revealed a normal optic disc and normal retinal vessels The macula in each eye showed an atrophic pigmentary lesion with irregular margins Surrounding the macula were many yellow white flecks (Fig 4 A) Angiograms showed numerous hyperfluorescent areas with abolished visibility of the chorioidal circulation (Fig 4 B and Fig 4 C)

*Case 3 (II 1)* A 20 year old man the youngest brother of case 1 noticed decreased central vision in both eyes at the age of 13 years Visual acuity was 6/10 in the right eye 6/20 in the left eye ERG and dark adaptation were normal He identified only the first of the Ishihara plates and a central scotoma was present in each eye In the macula most pronounced in the left one were some deepseated yellow white flecks

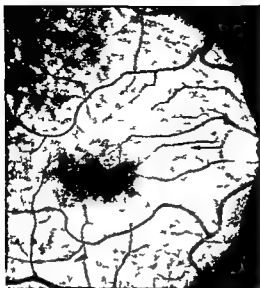


Fig. 2 A

Case 1 19 3 Right eye A An atrophic perifoveal lesion surrounded by numerous flecks B Angiogram (preretinal phase) showing numerous hyperfluorescent areas

Angiogram (arteriovenous phase) showing areas of hyperfluorescence of greater intensity than in B but the shape size and location are identical (Vertical arrows points to identical hyperfluorescent areas The long horizontal arrow demonstrates a retinal fleck showing fluorescence The short horizontal arrow demonstrates non fluorescent flecks) Note the abolished chorioidal circulation between the hyper fluorescent areas



Fig. 2 B



Fig. 2 C



Fig 3 A

Fig 3 B

Case 1 19/6 Right eye A A marked atrophic lesion and numerous fleck areas  
 B Angiogram (arterio-venous phase) showing more fluorescent areas than Fig 3 A  
 Since 1963 a fleck has become non fluorescent (long horizontal arrow) and a previously  
 non fluorescent fleck now shows fluorescence (short horizontal arrow)

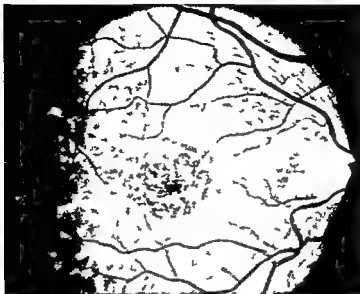
Most of them were round others with a more linear shape (Fig 5 A) The angiogram (Fig 5 B) showed no hyperfluorescent areas and no visibility of the chorioidal circulation

In these three cases a diagnosis of fundus flavimaculatus was made The siblings were also examined All had normal retinal findings and normal fundus angiography (Fig 6)

Case 4 A 24 year old woman in good health with no eye disorders in her family noticed reduced central vision in both eyes at the age of 12 years Visual acuity 6/40 in each eye ERG and dark adaptation were normal She identified only the first of the Ishihara plates The macula in each eye showed an atrophic pigmented lesion with beaten margins No flecks were seen (Fig 7 A) The angiogram showed almost no chorioidal flush and a hyperfluorescent macular area (Fig 7 B)

Case 5 A 46 year old man in good health with no eye disorders in his family noticed central vision of the age of 29 years Visual acuity was 4/40 in each eye central scotoma was found in each eye In each macula an atrophic lesion with beaten margins was seen surrounded by some yellow white flecks Some of them were linear or pisciform with indistinct margins and a tendency towards fluorescence No flecks were seen outside the main vessels trunks (Fig 8 A) The angiogram showed abolished visibility of the chorioidal circulation hyperfluorescent macular area and hyperfluorescent areas in the macula (Fig 8 B)

In the last two cases a diagnosis of Stargardts disease was made

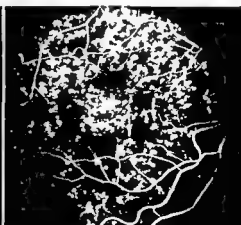


*Fig 4 A*

se 2 Right eye *A* An atrophic macular lesion surrounded by numerous flecks  
 Angiogram (preretinal phase) showing numerous hyperfluorescent areas *C* Angio  
 um arterio venous phase) showing areas of hyperfluorescence of greater intensity  
 in in *B* but the shape size and location are identical (Vertical arrows points to  
 ntical hyperfluorescent areas) Note the abolished chorioidal circulation between  
 the flecks



*Fig 4 B*



*Fig 4 C*





Fig 5 A

Fig 5 B

Case 3 Left eye A In macula some deeply seated retinal flecks are present. B Angiogram (arteriovenous phase) showing complete abolished visibility of the choroid circulation but normal filling of the retinal vessels

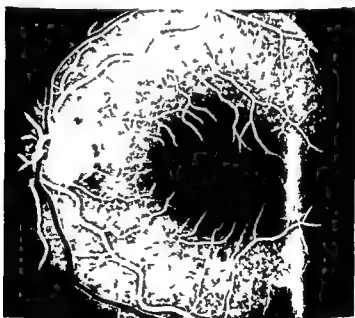
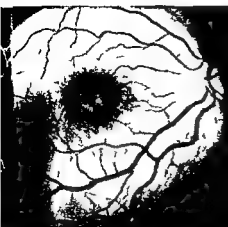
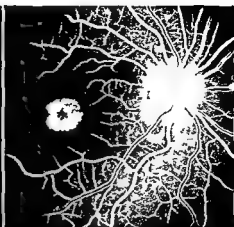


Fig 6

A healthy brother (II 4) in the family with fundus flavimaculatus Left eye Angiogram (early arteriovenous phase) showing normal angiographic pattern



*Fig 7 A*

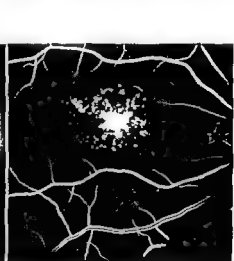


*Fig 7 B*

Case 4 Right eye *A* An atrophic macular lesion with beaten margins no flecks  
*B* Angiogram (arteriovenous phase) showing hyperfluorescence of the macular lesion  
 and normal filling of the retinal vessels There is complete abolished visibility of  
 the choroidal circulation



*Fig 8 A*



*Fig 8 B*

Case 5 Right eye *A* A macular atrophic lesion with beaten margins surrounded by  
 some flecks *B* Angiogram (arteriovenous phase) showing fluorescence of the atrophic  
 lesion and some of the flecks There is normal filling of the retinal vessels  
 but complete abolished visibility of the choroidal circulation

## Results

### Changes in the chorioidal circulation

The three members of the family with fundus flavimaculatus demonstrate a peculiar abnormal angiographic pattern: abolished visibility of the chorioidal circulation. This is most pronounced in case 3 (Fig 5 B) which is a very early stage of the disease. We also observed the same change in the chorioidal circulation in case 1 (Fig 2 C) and case 2 (Fig 4 B) although the marked hyperfluorescence tends to mask it. But between the hyperfluorescent areas there is no visibility of the chorioidal circulation. Case 4 and case 5 which fulfill the criteria of Stargardts disease also show the same invisibility of the chorioidal circulation (Fig 7 B and Fig 8 B). The siblings not affected by fundus flavimaculatus had a completely normal chorioidal pattern although all were haired with the same degree of retinal pigmentation (Fig 6).

### Hypo hyperfluorescence of the retinal flecks

In the very early stage of fundus flavimaculatus only some minor yellow white flecks are seen in the macular area (Fig 5 A). The angiograms demonstrate very few changes except the missing chorioidal flush (Fig 5 B). As the disease progresses more flecks are seen and the angiograms now reveal numerous hyperfluorescent areas. The flecks are usually found to be non fluorescent and the fluorescent areas in the angiograms are either seen between flecks or adjacent to the retinal flecks.

The retinal flecks are often found to fade away (Fig 2 A and Fig 3 A). Corresponding to the previous fleck a hyperfluorescent area is now formed. Since the retinal flecks may fade away and the hyperfluorescent areas persist a disproportion is made between the number of retinal flecks and hyperfluorescent areas. In some few cases we have seen a hyperfluorescent area to disappear but this is unusual (Fig 2 C and Fig 3 B).

The areas of hyperfluorescence are seen before filling of the retinal arteries both in fundus flavimaculatus and Stargardts disease. They have identical shape, size and location to those seen in the arterial and arteriovenous phases of the angiograms although the intensity increases (Fig 2 B and Fig 4 C, Fig 4 B and Fig 4 C). In no cases was late staining seen.

## Discussion

The abolished visibility of the chorioidal circulation is a peculiar in our patients with fundus flavimaculatus and Stargardts disease. The siblings not affected by fundus flavimaculatus all had a normal angiographic pattern.

ndus flavimaculatus and in Stargardts disease some alteration must exist with regard to the chorioidal circulation. The angiographic pattern could either be due to occlusion of the chorioidal circulation or something in front of the choriocapillaris that could obstruct the view of an otherwise normal chorioidal circulation.

In the preretinal phases of the angiograms numerous hyperfluorescent areas were seen. The location, size and configuration were identical to those seen in the retinal phases of the angiograms. The fluorescent areas increased in intensity during the arterial and arteriovenous phases and faded away simultaneously to normal chorioidal fluorescence. Between the hyperfluorescent areas no sign of chorioidal fluorescence was seen. The angiographic pattern of the hyperfluorescent areas in the preretinal and retinal phases are highly suggestive of normal chorioidal circulation which is seen through window defects in the retinal layers. The invisibility of the chorioidal circulation is probably due to a masking effect between chorioidea and the retinal vessels. The chorioidal circulation is obviously present but is only partly seen through the window defects.

As far as I know only Bonnin et al (1946) have stressed similar alterations in certain posterior retinal degenerations and have called it *le signe du fond choroïdien*.

Klien & Krill (1976) made the only histopathologic examination of an eye with fundus flavimaculatus. They found distinctive changes in the pigment epithelium while neuroepithelium, Bruchs membrane and chorioid were normal. The lesion appeared to be produced by an acid mucopolysaccharide located inside the pigment epithelium. The morphologic changes with displacement of the nucleus toward the center of the cell and the peculiar line of condensation of pigment granules near the inner surface of the cell can possibly explain increased absorption of the emitting and exciting light. A diffuse deposit of acid mucopolysaccharide will further increase absorption of the light.

The abolished visibility of the chorioidal circulation may thus be due to physical problems concerning the filtering of the emitting and exciting light. It is highly suggestive that a diffuse deposit at the level of pigment epithelium, Bruchs membrane or the outer segment of the retina blocks the transmission of light.

The missing chorioidal flush is not a constant sign in these two retinal diseases (Bonnin et al 1946). The reason for this is uncertain but the sign may be related to various amounts of diffuse deposits in the retina.

New flecks are usually non fluorescent. As the disease progresses they become fluorescent. The location, size and shape of the hyperfluorescent areas strongly suggest a pigment epithelial window defect.

Some of the flecks has been shown to fade away. We have also found earlier hyperfluorescent areas can be non fluorescent although most of them persist. The reason for this is uncertain.

The similar angiographic picture with abolished visibility of the choriocirculation and time related angiographic changes of the retinal flecks may be a further evidence that fundus flavimaculatus and Stargardt's disease are the same disease with a different expression.

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# RETINAL DAMAGE EXPERIMENTALLY INDUCED BY MICROWAVE RADIATION AT 55 mW/cm

BY

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and J SJÖSTRAND<sup>3)</sup>

The action of 500 W/m<sup>2</sup> (mean) 3100 MHz pulsed radiation on the rabbit retina *in vivo* was investigated by fundus photography blood retinal barrier tracers light and electron microscopy either after a single 1-1.5 h exposure or after a series of repeated 1 h exposures for up to 23 h during about 100 days

The electron microscopic investigation of the repeatedly exposed retinas revealed degenerative changes in the retinal neurons The neurons appeared depleted of their cytoplasmic constituents and often contained phago lysosomal structures with myelin bodies There were many degenerating synaptic boutons The glial cells displayed reactive changes These ultrastructural changes could not be demonstrated by the other methods used A single microwave exposure followed by an induced 10-10% blood pressure increase did not enhance blood retina barrier permeability to tracers There was no evidence of blood brain barrier leakage These studies show that the rabbit eye can be affected by microwave radiation at intensities lower than previously reported

**Key words:** retinal damage - degeneration of synapses - microwave radiation - brain - blood retina barrier - blood brain barrier - ultrastructure - fundus

The eye is one of the most well studied biological systems regarding the action of microwave radiation In clinical reports lens cataracts have been described as the most frequent hazard of microwave exposure in man Most experimental

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work on the effect of microwave radiation to the eye have been focused on induction of lens cataracts. The action of microwaves on the posterior of the eye has received less attention. Dailey et al (1948) reported retinal changes in dogs after high intensity exposure.

In a clinical report in 1952 Hirsch (1970) described a man developing cataracts, chorioretinal lesions and a scotoma following short term exposure to high intensity microwave exposure. Recently Aurell & Tengroth (1973) reported high incidence of retinal changes among the personnel of a radar industry.

Since our knowledge concerning the effect of microwave radiation on retina is very limited we have studied in the present work the action of 3 MHz pulsed microwaves on the rabbit retina. A number of experimental methods were used to investigate the retinal morphology *in vivo*, the retinal histology and the blood brain and blood retina barriers (BBB and BRB respectively) on animals subjected to either a single 1-1.5 h microwave exposure or a series of one h exposures repeated up to 53 times during a three month period.

## Materials and Methods

### Animals

40 pigmented rabbits of the black and white Dutch breed of both sexes weighing 1-1.5 kg were used. The microwave irradiations and the *in vivo* examinations were all performed without the use of any anaesthetic drugs.

### Microwave radiation

The microwave radiation was generated by an S band radar transmitter using a magnetron capable of delivering a peak power of 250 kW at the frequency 3100 MHz. The magnetron was coupled to an (IEC) R 32 waveguide which was terminated by a horn antenna with aperture dimensions 157 x 135 mm in the E and H plane respectively (E plane horizontal).

A movable short was used to match the magnetron to the waveguide for obtaining high peak power and a reasonably good pulse shape. The pulse length was fixed at 14  $\mu$ s and the pulse repetition frequency was 300 Hz. The mean power output was limited to 100 W by the power supply. All irradiations were performed at a distance of 0.5 m between the rabbit head and the antenna aperture. This is more than Fresnel distances to secure for far field conditions. Walls of microwave absorbing material was surrounding the rabbit at a distance of 1 m. The rabbits were irradiated from the right side with the head in the center of the beam. They were kept in a cage made of a polystyrene lattice and cooled by an air stream (2.5 m/s measured at the position of the head) from a fan situated well outside the microwave beam.

### Dosimetry and temperature measurements

The mean output power of the transmitter was measured to 100 W. The properties of the antenna was determined separately and the microwave intensity at the dis-

5 m was calculated to 500 W/m. The intensity could not be measured directly with power density meter because of the high pulse power. Calorimetric measurements in an ethanol filled spherical absorber calibrated against a power density meter gave a result an intensity of 500 W/m. A safe estimate of the mean intensity used would be 500 W/m  $\pm$  20% with a pulse peak intensity of  $1.5 \times 10^6$  W/m.

The temperature increase in the rabbit eye orbit during the irradiation was determined in a separate experiment where the rabbit was given a light barbiturate anesthesia and a few drops of oxibuprocaine (0.4%) as local anaesthetic. The rabbit was adapted for five min and thereafter the radiation was stopped. A miniature thermocouple was immediately inserted below the eye bulb in the eye orbit beneath the center of the eye and the temperature was registered. The irradiation was then restarted. This process was repeated for 60 min. The mean of measurements on two rabbits is given in Fig. 1.

#### Fundus photography (A)

The rabbits irradiated repeatedly were examined once a week by fundus photography (Table I). The central part of the retina was photographed with a 35 mm single lens reflex camera adopted for macrophotography with a long extension tube and an m

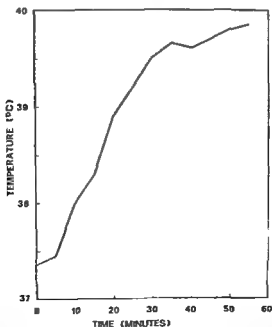


Fig. 1

Temperature increase in rabbit eye orbit below the eye bulb during microwave irradiation (31 GHz 500 W/m). The absorbed mean power density in the retina was about  $5 \times 10^5$  W/m<sup>2</sup>. Mean value of measurements on two rabbits.



Table 1  
Experimental conditions

Experimental method	Exposure conditions			
	I	II	III	IV
A <i>In vivo</i> retinal morphology fundus photography	✓			
B Light microscopy	✓			
C Electron microscopy	✓			✓
D BBB inspection of brain	✓	✓	✓	
E BRB fluorescence microscopy			✓	

I Repeated exposures 1 h/day 3 d/week up to about 30 h

II Acute 1 h exposure

III Acute 1 h exposure followed by a drug induced 70–100 % blood pressure increase

IV Acute 1 h exposure killed after 3½ months

verted standard lens. A small electronic flash illuminated the eye via a mirror outside the lens aperture. The refraction of the cornea-air surface was eliminated by a contact lens with a plane front surface (Goldmann lens, Haag Streit AG, Bern, Switzerland). The animals were given a few drops of Cyclogyl® (1 %) to dilate the pupil and oxibuprocaine (0.4 %) as a local anaesthetic. A small amount of methyl cellosolve secured good optical contact between lens and cornea. The magnification of the camera was fixed at 5×. The resolution enabled observation of single bundles of myelinated nerve fibres. Photographs were taken on 50 ASA colour slide film and viewed in parallel slide projectors by the investigators and others.

### Histology (B–C)

Animals in groups I and IV (Table 1) were killed and fixed by perfusion under pentobarbital anaesthesia. As a fixative we used glutaraldehyde and formaldehyde in cacodylate buffer as described by Karnovsky (1965). After perfusion 1 ml of the fixative was slowly injected into the vitreous body of each eye. An equal amount of fixative was allowed to drain from a small incision at the corneal margin. The eyes were further fixed by immersion. Parts of the central retina were postfixed in 1 % osmium tetroxide, dehydrated and embedded in Epon®. For light microscopy 1 µm sections were cut and stained with methylene blue. For electron microscopy 1 µm sections were cut with a diamond knife, further stained with uranyl acetate and lead citrate and observed in an electron microscope.

#### **Food tissue barrier studies (D E)**

Evans blue and sodium fluorescein were used as tracers in blood barriers permeability studies. Evans blue is strongly bound to serum albumin and the leakage of this dye through the blood retina or blood brain barriers indicates barriers permeability to molecules of high molecular weight. Sodium fluorescein is more loosely bound and dye penetrates more freely due to its lower molecular weight.

In most experiments Evans blue (20 mg/kg) was injected as a 2% solution in saline filtered through a 0.47  $\mu$ m Millipore® filter. The injection was given 30 min following start of the 15 h exposure. The animals were killed and fixed by perfusion under pentobarbital anaesthesia 70 min after the injection of the dye. 4% formaldehyde was used as a fixative. In those cases where sodium fluorescein was used the dye was injected (25 mg/kg) as a 10% solution immediately after the irradiation and the animal was killed by an overdose of pentobarbital after 15 min without perfusion.

The brain was dissected and the brain surface was inspected for the presence of dye leakage. In the case of sodium fluorescein the inspection was made under ultraviolet illumination.

All animals in the acute exposure group III (Table I) were subjected to an acute hypertension induced by an injection of metaraminol as it was possible that some animals might have experienced extreme stress accompanied with a hypertension during radiation and handling. Such acute hypertension was shown by Blomstrand et al (1970) to cause blood brain barrier leakage in X-ray irradiated rabbits.

The blood pressure was measured electromanometrically through a catheter inserted to the femoral artery. The catheter was inserted under light pentobarbital anaesthesia. During the surgery xylocaine® (1%) was given as a local anaesthetic. The animals were allowed to recover from the anaesthesia and were able to sit normally during irradiation. The dye and the metaraminol (0.15 mg/kg) was injected immediately after the irradiation.

Fifteen min after the blood pressure increase the animals were sacrificed and the brain surface inspected as described above. The right eye was enucleated and immediately frozen in liquid propane transferred to liquid nitrogen and freeze dried vacuum embedded in paraffin and sectioned for microscopy as described by Grayson & Bates (1971). The sections were observed in a fluorescence microscope.

## **Results**

The results are summarized in Table II.

#### **General observations**

The animals were checked regularly and showed no changes in weight appearance or motility as compared to the controls. The pupillary reflex was checked and the lens examined with a standard slit lamp. No difference between control and irradiated animals was seen. The appearance of the small vacuoles naturally located near the posterior sutures of the rabbit lens did not change during the repeated exposure experiment.

Table II

Frequency of positive findings in rabbit retina and brain with different exposure methods (A-E) after acute (II-IV) or repeated (I) 550 W/m<sup>2</sup> 31 GHz microwaves exposures J means irradiated groups and C non irradiated controls

Method	Exposure							
	I		II		III		IV	
	J	C	J	C	J	C	J	C
A <i>In vivo</i> retinal morphology fundus photography	0 <sup>1)</sup>	0						
	4	4						
B Retinal histology light microscopy	0	0						
	3	2						
C Retinal histology electron microscopy	3	0						
	3	2						
D BBB inspection of brain	1	1	4	1	0	0		
	3	2	9	7	3	9		
E BRB fluorescence microscopy					0	0		
					5	3		

I-IV For explanation see Table I

<sup>1)</sup> The figure 0/4 means that in a group of 4 animals 0 showed any changes

Table III

Thickness of some retinal layers in two different positions in microwave irradiated and control rabbits The values are given in  $\mu\text{m} \pm$  (standard error the mean  $\pm$ )

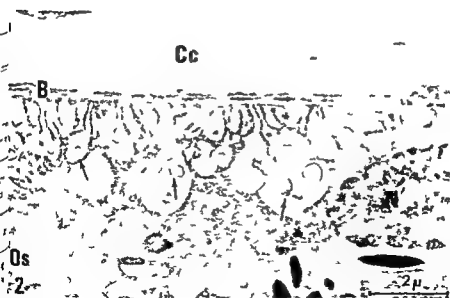
		Irradiated animals		Control
		right	left	
Photoreceptor outer segments	pos 1	39 $\pm$ 4	40 $\pm$ 4	33 $\pm$ 2
	pos 2	24 $\pm$ 1	24 $\pm$ 1	24 $\pm$ 1
Photoreceptor inner segments	pos 1	18 $\pm$ 1	18 $\pm$ 1	15 $\pm$ 1
	pos 2	17 $\pm$ 1	22 $\pm$ 3	14 $\pm$ 1
Outer nuclear layer	pos 1	56 $\pm$ 1	55 $\pm$ 1	64 $\pm$ 1
	pos 2	45 $\pm$ 3	47 $\pm$ 1	44 $\pm$ 1
Inner retina extending from the outer plexiform layer to the internal limiting membrane	pos 1	95 $\pm$ 5	103 $\pm$ 5	104 $\pm$ 1
	pos 2	83 $\pm$ 1	89 $\pm$ 4	84 $\pm$ 1

# fundus photography (A)

The fundus photographs from the repeatedly exposed animals were examined in pairs with the aid of two slide projectors. No signs of progressive changes in the retina were observed.

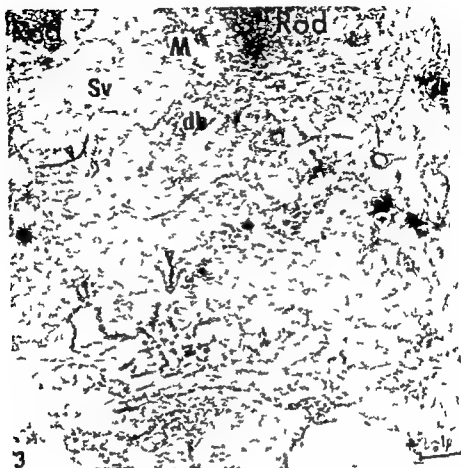
## Electron microscopy (B)

Electron microscopy of three different parts of the retina did not reveal any observable differences between the irradiated animals and the controls. The thickness of some clearly definable retinal layers was measured (Table III). No differences between the right and left eyes in the irradiated group or between the mean values of all eyes in the two groups are all well within 95% confidence intervals obtained from the *t* distribution. This result indicated for example that the processes of renewal and phagocytosis of the photoreceptor outer segment were not significantly affected by the radiation.



**Fig. 2**

Electron micrograph of pigment epithelial cells (N) from a rabbit repeatedly exposed during 3 months to microwave irradiation. There is an increased accumulation of membranous material (arrows) in the dilated spaces between basal processes of the epithelial cells and the basement membrane (B) facing the choriocapillary layer (Cc). Outer segments (Os) of photoreceptor cells in the lower half of the figure. 10 000 $\times$ .



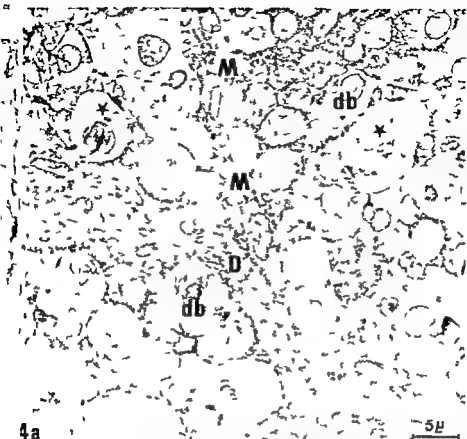
*Fig. 3*

Electron micrograph of the outer plexiform layer of rabbit retina repeatedly irradiated during 3 months. The enlarged synaptic terminals are distorted and contain numerous vesicles (Sv). Synaptic ribbons are marked by arrow heads. The Mullerian (M) neuroglial cells form thin multiple lamella (arrows). Dense bodies (db) = dense bodies. Rod = rod cell. 16000x.

*Fig. 3 a and b*

*4a* Electron micrograph of the same retina as in Fig. 3. Dense bodies (db) containing myelin figures are seen in synaptic terminals with only a few microtubules and filaments retained. There is an accumulation of fuzzy material (\*) in several terminals. A synaptic junction is marked by arrows. 2400x.

*4b* Electron micrograph of rabbit retina repeatedly microwave irradiated for 3 months. The synaptic terminals in the inner plexiform layers contain a large number of synaptic vesicles (arrow heads). There are two dense bodies containing myelin figures (db) in the synaptic terminals to the left. A synaptic connection is seen in the upper right. The Mullerian cell (M) shows reactive increase of glycogen and glycogen granula. 14000x.



4a

5μ



4b

1μ

# Electron microscopy (CI)

The electron microscopic investigation of the central retina in the rabbit three repeatedly irradiated rabbits revealed a spectrum of changes. There was an increased spacing between the basal processes of the pigmented epithelial cells as well as a slightly increased accumulation of debris (Fig. 2). The photoreceptors appeared normal with regard to the structure of their outer and inner segments. The synaptic terminals in the outer plexiform layer were dense and markedly increased in size (Fig. 3). Many of them contained very few synaptic ribbons and numerous dense bodies (Fig. 3). The enlarged synaptic terminals were filled with synaptic vesicles and contained only few other organelles. Multiple layers of glial membranes were often observed between these synaptic terminals. However, in contrast the synapses in the inner plexiform layer contained a reduced number of synaptic vesicles (Fig. 4). Dense bodies, sometimes including myelin figures, were frequently observed between synaptic terminals and neuronal processes (Fig. 4). The number of microtubules and to a smaller extent filaments were reduced. Several axons and dendrites contained fuzzy material (Fig. 4).

Many of the ganglion cells showed an increased number of dense bodies as well as of large vacuoles and of vesicles filled with fuzzy material (Fig. 5a). There was a loss of ribosomes, profiles of endoplasmic reticulum and mitochondria too. Spindle shaped dilatations of axons originating from ganglion cells were frequently observed as were similar axonal dilatations (Fig. 5a). Prominent gliotic reactions of mainly Mullerian neuroglial cells were seen in all layers of the retina, especially at the posterior pole of the eye (Figs. 3-5). There was an increased number of filaments, ribosomes and glycogen granules in the glial processes, which sometimes formed multiple sheaths around the neuronal processes (Figs. 3 and 4).

Thus, there were extensive changes in the majority of the neuronal processes in the plexiform layers, although there was a wide range in the degree of

*Fig. 3 a and b*

5a Electron micrograph of the ganglion cell layer of rabbit retina in Fig. 1. The ganglion cell (Gr) contains a vesicle filled with fuzzy material (+). The neuroglial cells (M) are reactive and filled with glycogen granules and filaments. Some axons to the left (arrows) are dilated and filled with fuzzy material (\*). 10 000 ×

5b Higher magnification of the nerve fibre layer adjacent to the vitreous body of the retina in Fig. 3. Enlarged axons (arrows) filled with dense bodies (db) and filamentous material (f) are seen. The axon (\*) above is also enlarged but does not display any true change in organelle distribution. Mullerian cell process (M), forms complex pattern close to the basement membrane (bm). 22 000 ×





damage. The most extensive changes were observed at the posterior pole. However, it should be stressed that many neuronal processes and cell bodies showed significant changes.

The left eyes of the animals irradiated from the right side and therefore having received less radiation were also examined. The retina of these eyes showed changes as described above. However, these changes were not extensive but had to be considered significant in a subjective evaluation.

Two rabbits (C IV) exposed once for 1 h and killed 3.5 months later showed changes in their right retinæ similar to those described above for the left eyes of rabbits subjected to multiple exposures.

#### **Blood brain barrier studies (DI–DII)**

The inspection for dye on the brain surface revealed some small (<1 mm) coloured spots on some of the brains. In the repeated exposure group (DI) one animal showed two such spots and in the control group one animal showed one spot. In the acute exposure group (DII) such spots were seen in 4 of 9 irradiated animals and in one of 7 controls.

From statistical point of view, no significant difference exists between irradiated and control groups at a 95% confidence level.

#### **Blood brain barrier studies on rabbits with acute hypertension (DIII)**

All animals in group III were subjected to a 70–100% blood pressure increase immediately following the irradiation and dye injection. No signs of dye leakage were seen in either control or irradiated animals.

#### **Fluorescence microscopy (EIII)**

About 7–10 sections from each right eye in the hypertension group were examined in the fluorescence microscope. The red fluorescence of the Evans blue dye or alternatively the yellow-green fluorescence of the sodium fluorescein dye was seen with extreme intensity in the choroid and with decreasing intensity towards the sclera. No traces of the fluorescent dye could be seen in the retinal layers internal to the pigmented epithelium. The sensitivity for detecting a weak fluorescence in the outer retina was somewhat lowered by the autofluorescence of the photoreceptors. It was possible to discriminate between the colour of this autofluorescence and that from the dyes by using different viewing filters in the microscope.

## DISCUSSION

threshold level of 1–1.5 kW/m<sup>2</sup> incident 2450 MHz microwave power for the formation of lens cataracts in the rabbit eye is now generally accepted. The electron microscopic examinations in the present work reveal significant retinal changes after repeated 0.55 kW/m<sup>2</sup> pulsed 3100 MHz exposure. The absence of lens opacities showed that the power level used is not substantially higher than the stated 0.55 kW/m<sup>2</sup>. These findings indicate that lesions observable at an electron microscopical level can be produced in the rabbit eye at power levels lower than the cataractogenic threshold. No retinal changes were seen, however, by light microscopy and no leakage of fluorescent tracer dyes through the blood retinal barrier was found. This agrees well with the fact that no changes could be observed with ophthalmoscopy and fundus photography. The report of Hirsch (1970) on ophthalmoscopically observable retinal changes in man intermittently exposed to 1–9 kW/m<sup>2</sup> during three working days shows that such changes may be caused by high intensity microwave radiation. The retinal changes on the personnel of a radar industry reported by Aurell & Tengroth (1973) were just detectable by a trained ophthalmologist equipped with good instrumentation (Aurell & Tengroth personal communication). The exposure history of these personnel was not very well known. Many of them had been working with microwaves for several years and it is likely that they were exposed to intensities well below 0.1 kW/m<sup>2</sup> for considerable times and that short exposures to much higher intensities occurred occasionally. It is reasonable to assume that the rabbits in the present work received microwave doses which fall between the two cases described above both in time and intensity. The frequency dependence of the absorbed microwave power in the human eye was investigated by Paulsson (1976). It is seen from that study that the human retina receives maximum power density at frequencies around 3000 MHz. For the rabbit eye, Guy et al. (1975) have found retinal power densities 4 times higher than that seen in the human eye, whereas measurements on a rabbit irradiated under our conditions gave about 2 times. Thus microwaves of frequencies around 3000 MHz are shown to be readily absorbed in the retina in both rabbit and human eyes. In the present work the absorbed mean power density in the retina was about  $3 \times 10^{-4}$  W/m<sup>2</sup>.

The ultrastructural examination of the microwave exposed retinæ revealed extensive changes both in synaptic terminals, neuronal cell bodies and processes as well as in neuroglial cells. A single exposure appeared to be sufficient to produce similar although much less extensive changes. Recently similar observations indicating damage of neurons in the brain were published by Albert & DeSantis (1975) and Switzer & Mitchell (1977). Hypothalamic neurons in brains

of adult hamsters displayed scarcity of rough endoplasmic reticulum and ribosomes as well as loss of microtubules. In both these two studies, the appearance of myelin figures in neuronal cells were frequently noticed as in retinal cells in the present study. There were also signs of loss of neurons in the exposed retinae. Thus the results obtained in the present study concerning damage by microwave irradiation are in agreement with those previously published.

We have also investigated the effect of 3100 MHz pulsed microwave microtubules and axonal transport (Paulsson et al. 1971). There were no effects at power levels comparable to or higher than those in the present work. The studies were restricted to acute exposures on *in vitro* systems and they are therefore not applicable to the now observed retinal changes after repeated exposures.

In the present work the brain received a microwave dose comparable to that of the retina. In the barrier studies no significant leakage of Evans blue 3 min through the blood brain barrier was observed even in animals which had induced acute hypertension. In contrast Frey et al. (1970) and Oscar & Perkins (1971) observed leakage of the blood brain barrier in microwave irradiated rats.

The mechanisms underlying the observed changes in the retina are not known. The temperature in the orbit beneath the eye was found to rise to 40°C during the irradiation period and the intraocular temperature was found to be 40°C near the retina 3 min after the irradiation. The action of some of the effects is therefore likely to occur. Whether non thermal effects are involved cannot be concluded.

In conclusion the present work has demonstrated that ultrastructural changes occur in rabbit retina repeatedly exposed to 0.55 kW m<sup>-2</sup> 3100 MHz pulsed microwave radiation. Further investigations should preferably be focused on the reversibility of these changes and the functional implications of these ultrastructural lesions.

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# INDOLEAMINE ACCUMULATING NEURONS IN THE RETINA OF CHICKEN AND PIGEON

A Comparison with the Dopaminergic Neurons

BY

I FLOREN

Recently a special group of indoleamine accumulating neurons has been described in the retina of some mammals and goldfish. These neurons are characterized by their ability to accumulate indoleamines whereby they become visible in the fluorescence microscope. They do not show any spontaneous fluorescence. The indoleamine accumulating neurons are in this study shown to be present in the retina of chicken and pigeon. Their cell bodies differ from the earlier described cell bodies of the same type in other species in being larger and bottle shaped instead of round or oval and in being situated further outwards in the inner nuclear layer. Their terminals ramify in three sublayers in the inner plexiform layer. No indoleamine containing neurons could however be seen to fluoresce in normal retina of chick embryos, newborn chicken, older chicken or pigeons.

**Key words:** Indoleamine accumulating neurons - dopaminergic neurons  
Retina - fluorescence microscopy - chicken - pigeon

Several of the so called biogenic monoamines (comprising catecholamines and indoleamines) are known or strongly suspected to be neurotransmitters in the brain, namely adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine. Of these, dopamine has been demonstrated in special neurons of the avian retina (Ehinger 1961; Stoeckel et al. 1966) as in many other species and it has generally been presumed that this was the only monoaminergic neuron type

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retina. However, lately a new set of neurons has been found in the retina of some species. They are characterized by their ability to accumulate indolamines avidly and can therefore be suspected to use such a substance as their transmitter. They are also distinguishable on morphological grounds and have far been demonstrated in rabbit, cat, goldfish (Ehinger & Floren 1976), river lamprey (Ehinger et al. 1977) and Cebus monkey (Dowling et al. 1979). The aim of the present study was to see if they are also present in the retina of birds and, if so, to describe their morphology. Special attention was paid to compare them with the dopaminergic neurons (which also have an uptake mechanism for biogenic monoamines and which could therefore be confused with indoleamine accumulating ones). Chicken were of particular interest because of a previous report of an indoleamine containing cell type appearing in the normal retina of chick embryos (Hauschild & Laties 1973). This special cell type was also searched for in the normal retina (i.e. without prior accumulation of exogenously applied indoleamines) of chick embryos as well as in that of the adult chicken.

### Materials and Methods

Chick embryos (White Leghorn) 13, 16 and 19 days; newborn chicken (White Leghorn) 1-2 weeks; chicken (Derko, a hybrid between Rhode Island and White Plymouthrock) 6-11 weeks; and adult wild pigeons (*Columba livia*) were used.  $\alpha$ -methylnoradrenaline HCl (Hoechst Laboratories), 5-hydroxytryptamine, 6-hydroxytryptamine (Sigma Chemical Co.) and 5,6-dihydroxytryptamine (Regis Chemical Co.)

*Table 1*  
Treatment scheme and number of eyes

Treatment	Chick embryos	Newborn chicken	Chicken 6-11 weeks	Pigeons adult
Normal	10	9	20	4
10 $\mu$ g $\alpha$ -methylnoradrenaline	-	2	10	4
10-20 $\mu$ g $\alpha$ -methylnoradrenaline	-	2	5	4
5-20 $\mu$ g 5-hydroxytryptamine	-	2	96	6
10 $\mu$ g 5-hydroxytryptamine or 10 $\mu$ g 5,6-dihydroxytryptamine*	-	-	-	-

\*On a molar basis 1  $\mu$ g  $\alpha$ -methylnoradrenaline equals 2  $\mu$ g 5-hydroxytryptamine or 5,6-dihydroxytryptamine.

all three obtained as creatinine sulphate were dissolved in 20  $\mu$ l 0.9% NaCl, ascorbic acid added (1 mg/ml) as antioxidant and injected intravitreally according to the treatment scheme given in Table I. Diamid<sup>®</sup> (Sigma Chemical Co) a metal oxidase inhibitor was given intraperitoneally in a dose of 100 mg/kg in two doses. The animals were killed by decapitating which in older animals was preceded by anaesthesia.

Three or four pieces of retina were taken at random four h after the intravitreal or intraperitoneal injection of the drug and were processed for fluorescence microscopy of indoleamines and catecholamines according to the method of Falck and H. In short the procedure involves freeze drying of the tissues exposing them to formaldehyde of controlled humidity and temperature whereby the fluorophores are formed. The tissues are then embedded in paraffin wax, sectioned and mounted. Catecholamines are characterized by their greenish and indoleamines by their yellowish fluorescence (filter settings most often used in the fluorescence microscope: blue is the true fluorescence colour of the fluorophore formed by catecholamines. The true colour of indoleamine fluorophore is yellow). For details of the histochemical procedure see Falck & Owman (1974) or Björklund et al (1972).

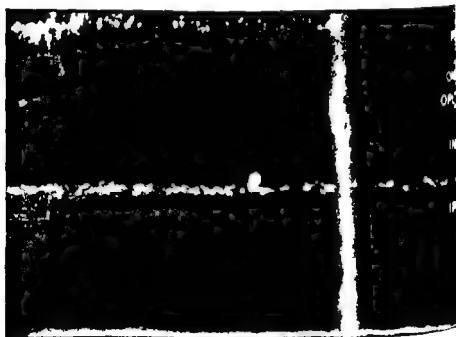


Fig 1

Normal retina from an adult pigeon fluorescence micrograph. There is one fluorescent cell body in the innermost part of the inner nuclear layer and three sublayers of fluorescent terminals in the inner plexiform layer. The innermost sublayer is very sparse. Ph photoreceptors. ONL outer nuclear layer. OPL outer plexiform layer. INL inner nuclear layer. ILL inner plexiform layer.  $\times 370$ .

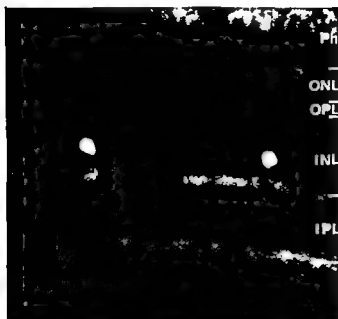


Fig 2

ina from an 11 week old chicken four h after the intravitreal injection of 20  $\mu$ g dihydroxytryptamine. Two bottle shaped cell bodies have appeared further out in the inner nuclear layer. The innermost sublayer of fluorescent terminals is now almost as prominent as the outermost sublayer. Fluorescence micrograph.

Designation of layer as in Fig 1  $\times 470$

## Results

### 1. retina

normal retina of chick embryos, newborn chicken, older chicken and treated according to the method of Falck and Hillarp contains fluorescent green round or slightly oval catecholamine containing perikarya in the cell row of the inner nuclear layer among the amacrine cells (Fig 1). They are the previously described dopaminergic neurons. Processes from these cells were not as readily detectable in the chick embryos as in the older chicken and the pigeons where they form three sublayers in the inner plexiform layers one at its outer border, one in its middle and one at its inner border (Fig 1). The outermost layer is most developed and the outer two are sparse. The innermost layer is in fact often hard to discern.

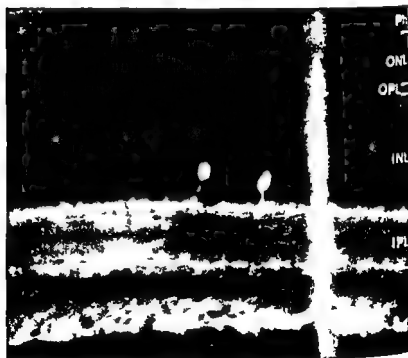


No yellow indoleamine containing cells can be seen with certainty in *in vivo* animals. Treating with Niamid® a monoamine oxidase inhibitor for 1-2 weeks intraperitoneally (100 mg/kg) enhances the catecholamine fluorescence by an additional structure becomes visible.

#### Retinas after the intravitreal injection of a catecholamine

Several attempts to inject substances into the vitreous of chick embryos were made but they survived for less than one h and results with drug injections in chick embryos are therefore not available.

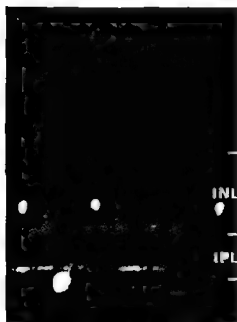
If small amounts (2 µg) of a catecholamine ( $\alpha$ -methylnoradrenaline) are injected into the vitreous of newborn chicken and older chicken a day or four h before processing the retina, there is a slightly enhanced green fluorescence of the structures described in the normal retina. Sometimes a new



**Fig 3**

Retina from an adult pigeon four h after the intravitreal injection of 20 µg 5-hydroxytryptamine. Two bottle-shaped cell bodies have appeared in the inner nuclear layer and the innermost sublayer of fluorescent terminals has increased to almost the same density as the outermost sublayer. Designation of layers as in Fig 1.

Fluorescence micrograph  $\times 400$



*Fig 4*

ina from a 6 week old chicken four h after the intravitreal injection of  $10 \mu\text{g}$  dihydroxytryptamine. Besides three bottle shaped cell bodies in the inner nuclear layer a fluorescent cell body has also appeared among the ganglion cells. Designation of layers as in Fig 1. Fluorescence micrograph  $\times 400$ .

of bottle shaped cell bodies situated two or three cell rows outwards in the inner nuclear layer were suspected.

With higher doses of the catecholamine ( $10\text{--}20 \mu\text{g}$ ) the bottle shaped cell bodies appeared clearly. They were seen to send processes to three sublayers of the inner plexiform layer, but now the innermost sublayer had increased in thickness and was about as pronounced as the outermost one.

With this higher dose ( $10\text{--}20 \mu\text{g}$ ) numerous bipolar like cells also appeared further outwards in the inner nuclear layer much like that seen with higher doses of an indoleamine. Their cell bodies are oval or round and smaller than the bottle shaped cell bodies and thereby readily distinguishable (cf Fig 5). Their processes reach both the outer and inner plexiform layers. These bipolar neurons are considerably more prominent in newborn chicken than in older chicken and pigeons where they are sometimes hardly demonstrable.

Retina after the intravitreal injection of an indoleamine

Injecting low to moderate doses ( $5\text{--}20 \mu\text{g}$ ) of an indoleamine (5-hydroxy-

tryptamine or 5-hydroxytryptamine or 5,6-dihydroxytryptamine) intravitreally in newborn chicken, older chicken and pigeons results in yellowish fluorescence after four h in perikarya in the innermost part of the inner nuclear layer. At the lower doses, however, these perikarya, which are the dopaminergic

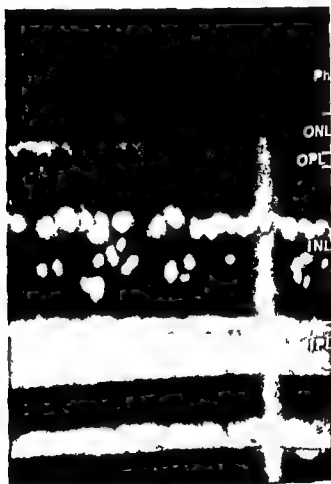


Fig. 5

Retina from a newborn chicken four h after the intravitreal injection of 5,6-dihydroxytryptamine. The same picture was seen with 10-20  $\mu$ g of methylnoradrenaline. The bottle-shaped cell bodies are seen in the inner half of the inner nuclear layer. Their processes reach the innermost sublayer. A number of bipolar cell bodies are seen in the outer half of the inner nuclear layer. Their processes extend outwards as well as inwards. In order to show these processes as well as possible, the details appear to be less distinct. Designation of layers as in Fig. 1.

Fluorescence micrograph  $\times 410$

are still of a more greenish colour. The number of the cell bodies in the innermost part of the inner nuclear layer is not increased as compared to the normal retina. Two or three cell rows outwards in the inner nuclear layer another group of neurons appear (Figs 2 and 3). They always displayed strong yellow fluorescence. The number of these neurons is two or three times as high as that of dopaminergic neurons. Their cell bodies are bottle shaped in contrast to the round or oval dopaminergic ones and have a considerably larger size. No significant difference in the appearance or number of the bottle shaped cells were seen between central and peripheral parts of the retina. Sometimes a few yellowish fluorescing cell bodies were seen in the ganglion cell layer (Fig 4). The processes of all these cells ramify in three sublayers in the inner plexiform layer. The outer and innermost sublayers are now about equally developed (Figs 2 and 3). The middle sublayer is poorly developed and therefore does not show up in micrographs.

Using the above amount (5-20  $\mu$ g) of an indoleamine also reveals a great number of yellowish fluorescing perikarya still further outwards in the inner nuclear layer with the same position, number and appearance as those of the above mentioned bipolar like cells also appearing after catecholamine treatment (Fig 5). Especially with the lower indoleamine amounts (5  $\mu$ g) the bottle shaped cell bodies have a much more intense yellow fluorescence than those of the bipolar like cells which with this dose are only faintly discernible. Increasing the injection amounts increases the fluorescence of the bipolar like cells but without reaching the same intensity as the bottle shaped cells. With indole doses higher than 25  $\mu$ g fluorescence appears in all parts of the retina. This is apparently an overdosage effect.

## Discussion

The occurrence of dopaminergic neurons in the retina is known in many species including chicken and pigeons (for reviews see Stell 1972, Graham 1973, Roach 1973, Ehinger 1976, 1979). The neurons which appear green in the fluorescence microscope all have their cell bodies in the innermost part of the inner nuclear layer among the amacrine cells. They send their processes to one or more sublayers in the inner plexiform layer. In chicken and pigeons the innermost sublayer is best developed as confirmed in this study.

A different group of indoleamine accumulating neurons which have their cell bodies at essentially the same position as the dopaminergic ones but with a different distribution of their processes has been found lately (Ehinger & Loren 1976). They are characterized by their ability to accumulate indoleamines

whereby they appear yellow in the fluorescence microscope. It could be that the reason why more cells appear after injection of an indoleamine is that there is a second type of amine accumulating neurons present in the retina. Instead it could be suspected that the previously known dopaminergic retinal neurons may not always all be visible in normal animals but are made so when forced to accumulate an indoleamine. However the comparison between different doses of a catecholamine and an indoleamine excludes this possibility because a low dose of the catecholamine will not label the indoleamine accumulating neurons whereas a comparable dose of the indoleamine will label them exclusively. Only with high doses will the substances enter both neuron types. The observations which are made in this study agree with previous findings in the rabbit and goldfish retina.

The morphology of the indoleamine accumulating cell bodies is quite variable in the species investigated so far but the spread of the processes has been found to be variable. Thus rabbit and cat have round or oval cell bodies of approximately the same size as the dopaminergic ones. They are situated at the junction of the inner nuclear layer and the inner plexiform layer. In goldfish the cell bodies are somewhat smaller and often situated slightly more peripherally in the inner nuclear layer than the dopaminergic ones. The sublayering can be best developed either in the outer (goldfish) or inner part (rabbit) of the inner plexiform layer or be diffuse as in cat (Finger & Floren 1976). In the present study which also reveals indoleamine accumulating neurons in chicken and pigeons they are however found to deviate from the earlier known type. Their cell bodies are situated further outwards in the inner nuclear layer. They are also considerably larger than earlier seen and bottle shaped with their necks looking inwards extending in processes forming three sublayers. The outermost and innermost sublayers are about equally developed with a faint sublayer between. This variation between different species in the morphology of indoleamine accumulating neurons is not surprising if comparison is made with the dopaminergic retinal neurons which show considerable variations not only in the distribution of their processes but also in the type of synaptic connections established in different animals (Dowling & Finger 1975, 1978; Dowling *et al.* 1979).

Ramon y Cajal in his classical studies described several types of amacrine cells in the chicken retina (Ramon y Cajal 1912) but none in very good agreement with the fluorescence microscopical picture. For instance the previously well known dopaminergic neurons in the retina do not correspond to any specific type of amacrine (Finger 1964). The same seems to be true for the indoleamine accumulating neurons. Among silver stained stratified amacrine cells it seems to fulfill very well the requirements of both being situated further outwards

cells in the inner nuclear layer and ramifying in three sublayers of the inner plexiform layer as the indoleamine accumulating neurons do

Hauschild & Laties (1973) reported a spontaneously occurring indoleamine containing cell in the retina of chicken embryos from the 14th day. This cell somewhat smaller than the dopaminergic one was observed in the inner nuclear layer over three of four cell rows from the inner plexiform - inner nuclear layer junction. The relationship between this cell and the indoleamine accumulating bottle shaped cell now described seems close. The reason why we have not been able to demonstrate any indoleamine fluorescence in the normal retina of chicken embryos or older chicken but only after the injection of amines is on the other hand not clear. It is however noteworthy that the cell found by Hauschild & Laties (1973) was observable only during a brief period of the development. It is possible that particularly favourable conditions with higher indoleamine concentration might occur during a certain stage of development in a breed of chicken used by Hauschild & Laties. Some support for this is found in the reports of rapid fluctuations in the activity of enzymes involved in the indoleamine metabolism and also in indoleamine content in the eye of embryonic and newborn animals. Thus Baker & Quay (1969) in the developing eye of the frog *Xenopus* at hatching found a decreasing and then an increasing activity of the enzyme forming 5 hydroxytryptamine 5 hydroxytryptophan decarboxylase (5 HTPD). In the whole mouse eye the activity of 5 HTPD and MAO (monoamine oxidase) rises in the middle of the first week post partum and then decreases (Smith 1973). 5 hydroxytryptamine and its metabolite 5 hydroxyindoleacetic acid (5 HIAA) are highest in the whole mouse eye the first day post partum and then decline rapidly in the course of a couple of days (Smith & Baker 1974). In chicken the MAO activity towards 5 hydroxytryptamine is low at the 12th day of incubation and then increases remarkably until the 18th day of incubation. Thereafter the activity decreases and 2 weeks after hatching the level is the same as measured in the adult (Suzuki et al 1977b). Further in quantitative studies we have measured a higher amount of 5 hydroxytryptamine in the retina of newborn chicken than in chicken of 11 weeks (Floren & Hansson 1978). The difference is statistically significant using Student's *t* test. Suzuki et al (1977a) reported even higher values in older animals but their figures are not directly comparable because their method of analysis was different.

When higher amounts of an indoleamine are injected another population of neurons than the bottle shaped ones appears in the outer part of the inner nuclear layer. Their size is smaller and their gracile processes extend both outwards and inwards in the retina. These characteristics are strongly suggestive of bipolar cells although the cell bodies of the supportive glial or Mul-

lerian cells are located approximately at the same level of the retina. The processes of the Mullerian cells are however coarse in contrast to the fine processes of the bipolar cells and the processes of the Mullerian cells cover almost the entire retina which neither the presently observed processes nor processes of the bipolar cells do. When using lower amounts of an indoleamine the bipolar like cells are hardly visible whereas the bottle shaped cells are very prominent. Using high amounts of a catecholamine also makes the bipolar like cells appear in chicken but only to a minimal extent in pigeon. As in chicken the fluorescence (i.e. the amine uptake) of the bipolar like cells is much stronger in newborn chicken than in chicken of 11 weeks. The transition into identical bipolar like cells of either an indoleamine or a catecholamine when used in high dose therefore seems to represent an uptake not necessarily related to any transmitter function of the accumulated substances. The fact that the uptake is higher in newborn than in older animals suggests an higher uptake into less differentiated bipolar like cells later undergoing specialization.

The present demonstration of indoleamine accumulating neurons in birds together with previous observations of apparently homologous neurons in mammals (Linger & Florén 1976; Dowling et al. 1979) goldfish (Chin et al. 1976) and river lamprey (Linger et al. 1977) suggest their widespread occurrence in the retina of vertebrates. The transmitter of the indoleamine accumulating neurons is however not known. The only indoleamine which is currently regarded as a neurotransmitter in the vertebrate brain is 5 hydroxytryptamine which can be seen in the fluorescence microscope. However we have not been able to demonstrate any neurons with the typical 5 hydroxytryptamine fluorescence in normal bird retinas. Similar observations have been made in mammals (Florén 1978). The reason for this could be a very low concentration of 5 hydroxytryptamine in the neurons but this seems somewhat unlikely because in other parts of the brain the 5 hydroxytryptamine neurons have sufficient transmitter concentration to make them demonstrable with the fluorescence microscope. Alternatively then the transmitter could be an indole other than 5 hydroxytryptamine and incapable of forming fluorophores with formaldehyde in the Falck and Hillarp histofluorescence method. There is indirect evidence that this may be the case in rabbits which will be discussed elsewhere (Florén 1978) and possibly this is also the case in chicken and pigeons.

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## TYPICAL AND ATYPICAL MONOCHROMACY STUDIED BY SPECIFIC QUANTITATIVE PERIMETRY

BY

EGILL HANSEN

Two blue cone monochromats and four rod monochromats have been studied by increment threshold measurements applying the Stiles principle. Some rudimentary colour discrimination was reported by the blue cone monochromats. One patient showed good discrimination between short and middle wavelength lights in matching experiments using the Nagel II apparatus. His neutral band in the spectrum was at  $\lambda = 485-495$  nm. Dichromatic vision could not be proved in the other patient.

The blue cone monochromats also had good responding blue mechanism in the periphery. Indication of cone activity other than blue cones is found in both kinds of monochromats: these cones being probably of the rhodopsin cone type ( $\tau_0$  cones). The conclusions are drawn that the inhibitory effect of the  $\tau$  cones upon the rod mechanism may account for the differences shown by our two blue cone monochromats as to visual acuity, nystagmus and photophobia. Likewise their differences regarding dichromatic vision may be explained by an unequal number of  $\tau$  cones in their retinas rather than by differences in their blue mechanisms.

*Key words:* blue cone monochromacy - rod monochromacy - colour vision - static perimetry - Stiles functions

An atypical form of monochromacy designated blue mono cone or blue cone monochromacy was first described by Blackwell & Blackwell in 1957. It differs from the typical rod monochromacy by the presence of functioning blue cones in addition to rod receptors. The condition is very infrequent. In a review by Alpern (1974) only 20 cases, all males, had been verified. By ordinary

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methods including the greater part of the colour vision tests and ophthalmoscopic examinations there may not be any characteristic differences between the typical and atypical monochromacies.

Perimetric registrations during selective chromatic adaptation (Hansen 1951) make it possible to study the receptor mechanisms separately by the central and peripheral response functions. It is the purpose of this study to report characteristic findings in persons who are blue cone monochromats and to compare them with the findings in typical total monochromats who have been examined with the same methods.

## Material

**Case 1 (BTW)** a 17-year-old male is a blue cone monochromat. There is consanguinity in the family and no other cases of amblyopia or other visual defects. He is the second child. The visual impairment had been present from birth. The patient had moderate photophobia in daylight. He remained blind in twilight. He confused yellow and green which however were distinguished from blue. Blue, violet and purple were confused like red, black and brown. He exhibited a coarse pendular nystagmus. Fixation was central. Haidinger's brushes could not be seen. Normal pupillary reactions were found. On ophthalmoscopy the fundus appeared normal including the optic disc and retinal vessels. Visual acuity was 6/60 on each eye. Normal visual field limits were found in the Goldmann perimeter (Object size 16 mm, 10 min arc intensity). His dark adaptation curve obtained in the Goldmann-Went adaptometer was subnormal, monophasic (final thresholds 1/4 log units above normal level).

**Case 2 (HII)** a 10-year-old male is a blue cone monochromat. The patient has always been partially sighted. A heavy jaundice had been noticed after birth. He was in good health. There was no consanguinity in the family and no other cases of amblyopia. He is the only child. The parents have been found normal by colour vision tests. His maternal grandfather, a protanope, was examined here. A brother of the grandfather is also said to be red-green deficient. The patient had no photophobia and performed well in twilight. There was a slight inconstant nystagmus. The pupillary reactions were normal. Ophthalmoscopy showed a normal fundus and a faint foveolar reflex. Visual acuity was 6/60 on each eye. Colour vision had always been poor. Yellow, his favorite colour, was the easiest to recognize but could sometimes be confused with green. He could also see red and black. Blue as a rule was not distinguished from violet, pink or purple. On the other hand, red was not confused with green or yellow.

The following four patients who were typical rod monochromats had not been able to distinguish colours. They all had some photophobia. *Case 3* (OF) 30 year old male who had moderate nystagmus like *Case 4* (ER) a female 30 years of age. *Case 5* (NN) a male 32 years of age who had very slight nystagmus. *Case 6* (OK) a male 35 years of age in whom no nystagmus could be seen. Visual acuity which was optimal at reduced illumination was 5/36 in cases 3 and 5, 6/20 in case 5 and 5/60 in case 4. They all had slight to moderate hypermetropia and a moderate regular astigmatism (cases 4, 5 and 6). Pupillary reactions were noticed (cases 3, 5 and 6). On ophthalmoscopy normal optic discs were found as well as normally calibrated vessels. In cases 3 and 5 the macula was poorly marked. Foveolar reflexes were not seen. ERG recorded in patient 6 showed a normal response to single flash stimuli under photopic conditions but no response to flicker at 25 Hz. The father as well as two brothers of patient 3 were rod monochromats. The parents were first cousins. Rod monochromacy was found in a brother and a sister of patient 5.

## Methods

The colour vision tests used comprise the Ishihara test (11th ed.) the AO HRR test, the Farnsworth D 15 test, the Farnsworth Munsell 100 Hue test, the Farnsworth 15 Munsell hue plate and the Sloan's achromatopsia test. A series of charts reproduced in various shades from charts 10-14 of the Ishihara test (Hansen 1963) were shown together with the pseudo isochromatic tests. The pigmentary tests were administered under Macbeth Illuminant C lamps at about 300 lux.

Anomaloscopic examinations were performed with a Nagel anomaloscope type I. Additional examinations were performed in the blue cone monochromats with a calibrated Nagel anomaloscope type II where colour equations could be tested also in the short wavelength part of the spectrum. The widest aperture of the apparatus was used (3.12 visual angle).

Static perimetry under varying adapting conditions was performed in a modified Goldmann perimeter as described earlier (Hansen 1964; Hansen & Seim 1968). Near monochromatic interference filters (half band width 10-15 nm) were used and in some experiments moderately narrow banded interference filters (half band width 30-47 nm). The angular size of the target is expressed in degrees and minutes. As the target is slightly elliptical the diameter of the equivalent circular target with the same area is indicated. The background illumination was measured with a luxmeter (Hartmann & Braun EBLX3) in the bottom of the bowl. The luminance of the test targets was calculated as the reflected energy at the level of the sphere. Fixation could be controlled by means of the telescope of the apparatus except for registrations done in total darkness. In cases of obvious nystagmus fixation was unstable making the statement of eccentric angles only approximate.

## Results

## Pigmentary tests

The pseudo isochromatic tests could not be read by any of the rod monochromats except for some caudal charts. The blue cone monochromats could see the hidden digits and the transformation pattern charts (mainly the red green alternative) of the Ishihara tests. At the AO HRR test patient 1 could see three of the blue yellow charts and one of the red green charts. Patient 2 could see all the blue yellow charts but one and four of the red green charts. A set of charts reproduced in greyish shades from the Ishihara test could be seen by the rod monochromats as well as by the blue cone monochromats. With Sloan's achromatopsia test a typical achromatic pattern was obtained from the rod monochromats and for patient 1 who could not however make a grey match with the yellow print. Patient 2 matched a dark grey (35) with the red print but did not make matches with the other colours. At the Farnsworth tritan plate the blue cone monochromats could see the green square and not the blue square whilst two rod monochromats were unable to see the square. Several performances with the Farnsworth D 15 test revealed confusions without exact protan or deutan axis but mainly in the red green region for the blue cone monochromats whilst the rod monochromats demonstrated a typical scotopic pattern (Fig. 1). Patient 2 had 244 error scores at the Munsell test. The pattern was moderately distorted with the least errors in the red yellow red and in the blue to blue green regions (a reversed tritan pattern).

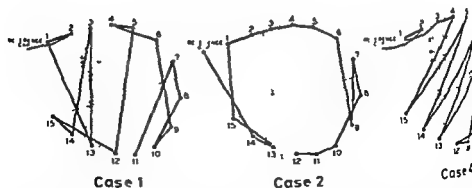


Fig. 1

Performance of two blue cone monochromats (Case 1 and 2) on the Farnsworth D 15 test compared with a typical performance of a rod monochromat (Case 4).

Table 1

performance in the Nagel anomaloscope type I of two blue cone monochromats (case 1-2) and 4 rod monochromats (case 3-6). The figures indicate the yellow values used to match the upper field (the red green values)

Case	Red green value						
	75	60	55	50	40	30	
1	$\frac{1}{2}-1$		$34\frac{1}{2}$		71		Not possible
2	$\frac{1}{2}-1$	25		$43\frac{1}{2}$	61	9	Not possible
3	$1-1\frac{1}{2}$				55		Not possible
4	1				54		Not possible
5	$\frac{1}{2}-1$	25			56		Not possible
6	1				$61\frac{1}{2}$		Not possible

#### Anomaloscope tests

With the Nagel anomaloscope type I a typical achromatic pattern was found in all the patients (Table 1). Matches all over the scale were accepted except for the green values which were too bright for matching with the yellow field. Red was matched with a very dark yellow.

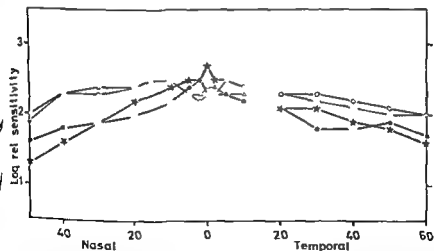


Fig. 2

static perimetry in white background (10 cd/m<sup>2</sup>) for two blue cone monochromats (● BTW ★ = HH) and two rod monochromats (○ = OF △ = ER). Angular diameter of target = 54°. Shaded area indicates the normal variation (mean ± 2 SD).

With the Nagel anomaloscope type II patient 1 showed an uncertain spectral hue in the region 480–505 nm while he indicated the sensation of colour for the shorter wavelength values and yellow for the longer wavelength values. However for a series of fixed settings of the main screw he accepted matches for wavelengths varying between 413 and 566 nm.

Patient 2 indicated a neutral spectral range at  $\lambda = 485$ –495 nm while he had the sensation of blue in the shorter wavelength region and yellow or green in the longer wavelength region. He could match the wavelengths 517 and 617 nm but did not accept any match for the wavelengths 485 and 517 nm or for the wavelengths 465 and 490 nm. The cyan blue equation  $\lambda_1 + \lambda_2 = \lambda_3$  was performed with a moderately small range of settings using the main screw (12–20 scale values).

### Quantitative perimetry

The perimetric profiles obtained under standard conditions are shown in Fig. 9. The curves are deflected and irregular in the central part for patient 1 (BTW) as well as for the two red monochromats Patient 2 (III) who had a better visual acuity. Patient 2 has a normal central profile though at a reduced level.

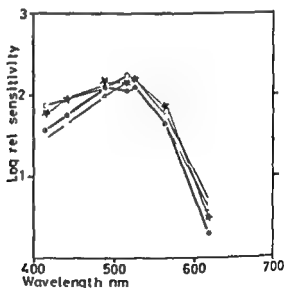


Fig. 9

Relative spectral sensitivity in white background (10 cd/m<sup>2</sup>) registered by use of moderately narrow banded interference filters. ● = BTW ★ = III Δ = OF. Angular diameter of target 54°. Shaded area indicates the normal variation (mean  $\pm$  2 SD).

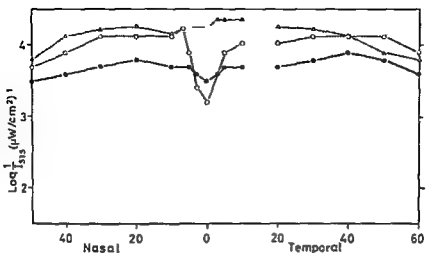


Fig. 4

Static perimetry during total dark adaptation recorded with green object light ( $\lambda_m = 515$  nm). Ordinate absolute threshold sensitivity for target subtending  $1^\circ$  angular diameter. ● = BTW ○ = OK △ = OF. Shaded area indicates the variation (mean  $\pm 2$  SD) for 3 normals.

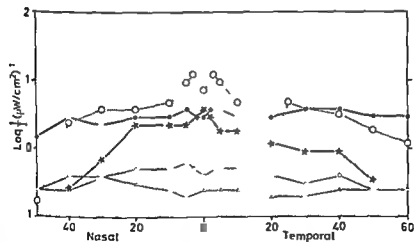


Fig. 5

Static perimetry in yellow adapting light ( $\lambda = 559$  nm, 9000 lux) registered with near monochromatic object lights. Blue violet light ( $\lambda_m = 459$  nm) is used in the registration of BTW (●) HH (★) and the normal observer (stippled line) and green light ( $\lambda_m = 500$  nm) in the registration of the rod monochromats OK (○) and OF (△). Angular diameter of target  $1^\circ 47'$ .



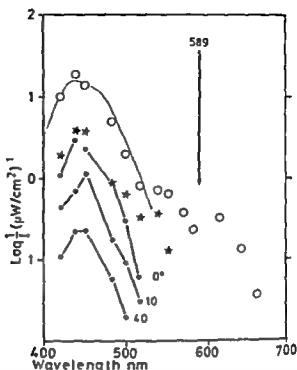


Fig 6

Spectral threshold sensitivity registered in BTW (●) IIII (★) and a normal person (○) under conditions as in Fig 5. The records of BTW are obtained centrally and at the perimetric angles 10° and 40° in the nasal field, the curves being displaced downwards and separated by  $\frac{1}{2}$  log unit. The thin line indicates the action spectrum of the blue mechanism (after Walraven 1974).

perimetric curves of the blue cone monochromats are still below the normal level in the periphery, while those of the two rod monochromats reach normal level. In the white background light (10 cd/m<sup>2</sup>) the spectral sensitivity curves are quite identical for the two types of monochromats with a maximum sensitivity at about 500 nm (Fig 3). Static perimetry performed during dark adaptation shows a quite flat perimetric curve for the blue cone monochromat case 1 as well as for the rod monochromat case 3, whilst the rod monochromat case 6 has a perimetric profile which is well fitted to the normal pattern (Fig 4).

In the bright yellow light the rod monochromats were not able to see short wavelength targets neither centrally nor peripherally. However, the red targets at  $\lambda_m = 500$  nm were easily seen and could be followed towards

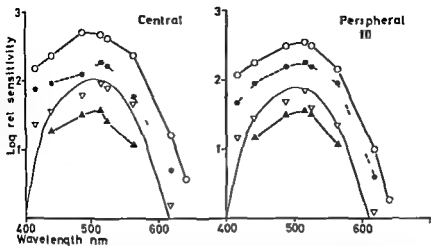


Fig 7

relative spectral sensitivity registered by use of moderately narrow banded interference filters in a rod monochromat (ER) in different adapting lights (from top to bottom) purple white yellow and blue A fitted scotopic luminosity curve (the C I E  $V_2$  curve) is indicated by the thin line

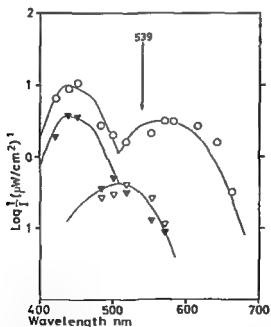


Fig 8

spectral threshold sensitivity in a near monochromatic green light ( $\lambda_m \sim 539$  nm, 30 lux) Angular diameter of target 1.47  $\blacktriangledown$  - BTW  $\triangledown$  - NN Open circles indicate response of a normal person Full lines indicate the blue and red mechanisms (after Walraven 1974) and the C I E scotopic luminosity curve (lowest)

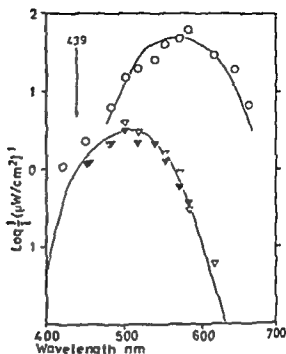


Fig. 9

Spectral threshold sensitivity in a near monochromatic blue violet light ( $\lambda_n = 439$  nm, 1.3 lux). Explanation as for Fig. 8.

the periphery (Fig. 5). The curves for the rod monochromats expressing the peak spectral sensitivity are at low levels. For the blue cone monochromats static perimetry with the object light at  $\lambda_n = 439$  nm could be performed, showing a clearly higher sensitivity, even though the sensitivity level in general does not reach that of a normal person. Spectral sensitivity curves registered under the same conditions are shown in Fig. 11. The peak sensitivities correspond with that of the blue cone mechanism. Patient 1 (BTW) has nearly identical response levels both centrally and at the perimetric angles 10° and 40°.

The typical rod monochromats show the same type of action spectrum irrespective of the quality of the background illumination. This indicates that only one receptor mechanism is present and responsible for the sensation. In two of the rod monochromats, case 3 and case 4, quite identical registrations were obtained centrally and at the 10° nasal position. Fig. 12 shows the results for one of the patients (case 4).

Fig. 8 shows the action spectrum of case 1, one of the blue cone monochromats, in a green background light as compared with the response of a

monochromat case 5 and a normal observer. The curves for the blue cone monochromat indicate the presence of two kinds of receptors which are in accordance with the  $\pi_1$  and  $\pi_0$  mechanisms of Stiles. Only one type of receptor mechanism is demonstrated in the rod monochromat. With a blue violet background light case 1 records an action spectrum which is in accordance with the  $\pi_1$  mechanism and is identical with the response of the rod monochromat (Fig. 9).

## Discussion

Measurements in the standard luminance of the perimeter ( $10 \text{ cd/m}^2$ ) our blue cone monochromats show a typical rod response pattern the same as that of the rod monochromats (Fig. 3). Blackwell & Blackwell (1961) found bimodal curves with peaks corresponding to the blue cone and rod mechanisms and so did Kornyn et al. (1970). In spite of several attempts Alpern et al. (1965) could not obtain a bimodal curve but they found in their blue cone monochromat that the luminosity curve was dominated by a pure rod curve or by a pure blue receptor curve depending on the background illumination. Registration of luminosity curves in white backgrounds therefore may not be a reliable method for separating the two types of monochromats.

The luminosity curves registered against the yellow background of the perimeter (Fig. 6) show a distinct blue receptor response in both our blue cone monochromats. The peak sensitivity which approximately is at the same level for the two patients is somewhat below that of the normal observer. The typical rod monochromats being unable to see the blue violet target at all but exclusively the 500 nm target have reduced sensitivity by at least 1 log unit in the blue spectral region in relation to the blue cone monochromats. Therefore a distinction between the typical rod monochromats and the blue cone monochromats can be obtained by perimetry using blue targets and a low pressure sodium lamp as the adapting light.

Blackwell & Blackwell (1961) in their blue cone monochromats found blue cone response only in the central visual field and stated that there was no evidence that a significant number of functioning blue cones exist at a retinal location 15° from the fovea. This is not in accordance with the present findings in our patients. The static perimetry curves obtained in bright yellow light indicate that there are also well functioning blue receptors in the periphery to the same extent as in normal individuals (Fig. 5). Even with the gross nystagmus displayed by patient 1 no response would be expected at the 40-60 perimetric angles if the blue cones were strictly limited to the central area. In both blue cone monochromats the perimetric curves expressing the blue mechanism

ism are somewhat reduced in relation to the curve of the normal observer. In patient 2 with a good central fixation it is noteworthy that the threshold sensitivity is highest at the fixation point and not paracentrally which is typical of normal individuals. This may indicate a blue cone distribution in the retina of patient 2 that is different from normal persons or else a possible lack of melanin pigmentation.

Blackwell & Blackwell (1961) suppose that in the mesopic zone where both the two receptor systems are acting the blue cone monochromats are blue cone rod dichromats. In the Nagel II anomaloscope patient 1 indicated a blue neutral band in the spectrum but his dichromatic vision could not be confirmed by the matching experiments. However in this case the examination was carried out with light of wavelengths only above 473 nm and his real neutral band might have been at shorter wavelengths. Patient 2 more confidently described a neutral band in the spectrum at  $\lambda = 485-495$  nm and his dichromatic vision was confirmed by the matching experiments. In this patient the exact equation ( $r_1 + r_2 = r_{10}$ ) was particularly good for proving dichromatic vision in the short wavelength part of the spectrum the lower reference field being coincident with his neutral point. This equation in the Nagel II anomalometer which was originally intended for testing tritanopes has been abandoned because of the different colour saturation in the two fields.

In her study of achromats Sloan (1955) postulated the presence of photoreceptors rods. Alpern et al (1971) suppose that the rudimentary colour vision of blue cone monochromats is mediated by blue cones and  $\tau$  receptors which are not rods but cones. They found that these receptors have the directional sensitivity and the dark adaptation curve of normal red and green cones. The  $\tau$  cones are not necessarily present in all blue cone monochromats. Daw & Easton (1973) examined one patient and did not find evidence for cones having a 500 nm pigment.  $\tau$  cones may be present in unequal numbers also in rod monochromats. For instance among our rod monochromats patient 6 (Ox) did not have nystagmus and had a relatively good central vision which might indicate a high number of  $\tau$  cones. His nearly normal perimetric profile obtained during dark adaptation (Fig. 4) may also indicate that his central receptors are of a kind different to ordinary rods.

Green (1972) found that the blue receptors in blue cone monochromats as well as in normal individuals contribute very little to the visual acuity. It is more uncertain to which extent the  $\tau$  cones may contribute to visual acuity. Possibly their influence on the visual acuity may be an indirect one due to the inhibition of the  $\tau$  rods under photopic conditions. This also might be an explanation for the differences in photophobia and nystagmus seen in monochromats.

Blue cone activity is considered to cause inhibition of the rod system. A reduced recovery rate of the rod mechanism after exposure to bright yellow light is seen in patient 1 (BTW) (Hansen et al 1978). Blackwell & Blackwell (1961) and photophobia, gross nystagmus and low visual acuity in their class III patients, presumably having the poorest blue receptor function. Grützner (1964) called attention to his atypical monochromats who showed inhibition of rod sensitivity under photopic conditions in spite of their loss of red and green cones. Trezona (1970) suppose that the inhibition of rods by cones is effected particularly by the blue cones. However the blue cones may not be the only inhibitor of rod activity in blue cone monochromats.

As our blue cone monochromats have good responding blue mechanisms as found by the chromatic adaptation studies and their threshold sensitivity is not particularly different, it is unlikely that their variance in visual acuity, nystagmus and photophobia is due to differences in their blue mechanisms. What is more likely is that the better performance of patient 2 under photopic conditions as well as his better colour discrimination and clear dichromatic matches in the short wavelength part of the spectrum is due to a higher number of  $\gamma$  cones in his retina.

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# INHIBITION AND FACILITATION OF LACRIMAL FLOW BY $\beta$ ADRENERGIC DRUGS

BY

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The effects of adrenergic  $\beta$  stimulating and  $\beta$  blocking compounds on tear flow were studied in conscious rabbits with a modified Schirmer technique. The tear flow was significantly increased by the unselective  $\beta$  adrenergic agonist isoprenaline and by the selective  $\beta_1$  agonist H 80/62 (racemic Prenalterol Hassle Sweden). The effects of the agonists were blocked by the unselective  $\beta$  adrenergic antagonist propranolol (Inderal®) and by the selective  $\beta_1$  antagonist metoprolol (Seloken® Lopressor®). The experimental data favoured the hypothesis that the  $\beta$  receptors involved in the regulation of the tear flow were of the  $\beta_1$  type. The anatomical location of these  $\beta$  receptors is at present unclear but *in vitro* experiments performed on the rabbit lacrimal glands indicated that the receptors were probably not located on cholinergic nerve endings as the release of acetylcholine was not influenced by isoprenaline.

**Key words:** tear flow – adrenergic betablockers – adrenergic betastimulators – animal experiments

■ reduction in tear flow was found in patients with conjunctival changes. In practolol (Eraldin® Eraldina®) (Wright 1978) it was of interest to study possible effects of other adrenergic  $\beta$  blocking agents – and especially  $\beta_1$  blockers – on tear flow. Earlier studies in animals (Botelho et al 1973 Boissier 1976) have indicated that stimulation of adrenergic receptors may increase lacrimal flow while adrenergic blocking agents have been reported to de-

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crease the tear flow. In this investigation we have tried to further elucidate the mechanism for the action of adrenergic  $\beta$  blocking agents on tear flow, and to determine which types of adrenergic  $\beta$  receptors that may be involved in these mechanisms.

## Methods

### I Experiments with conscious rabbits

The test of Schurmer (1903) and Norn (1944) was modified and a method, according to Bonnier et al. (1976) was developed for measuring tear flow in conscious rabbits. The rabbits were kept in ordinary laboratory rabbit cages. Their tear flow was measured with strips (3 × 40 mm) of filter paper (NIOR). A mark was made 5 mm from one end of the filter paper strip and the strip was applied to the rabbit's eye with the proximal 5 mm in the conjunctival sac and the remaining 35 mm hanging outside. The tear fluid was then taken up by the filter paper and after 60 sec the filter strip was pulled out. After a time lapse of another 5 sec during which tear fluid was descending along the strip, a second mark was made on the strip at the border between the dry and the tear wetted areas. This procedure was repeated every 5 min with new strips of paper for both eyes and the mean value of the distances between the 5 mm ink mark and the second mark was assumed to represent the tear flow.

When the tear flow had been stable for 15 min, saline or an adrenergic blocking agent was infused intravenously in a volume of 0.2 ml/min for 1 min via a marginal vein of an ear. Exactly 15 min after the termination of the intravenous infusion, 1 isoprenaline was given iv for 20 min via the same catheter. The dose of isoprenaline used was 0.3  $\mu\text{g/kg/min}$  in a volume of 0.2 ml of saline with ascorbic acid 0.1 mg/ml.

In experiments when isoprenaline was replaced by the  $\beta_1$  selective antagonist H 80/62 (racemic prenalterol, Carlsson et al. 1977) the latter was given in a dose of 60  $\mu\text{g/kg/min}$  in 0.2 ml/min of saline for 3 min. Thus the total dose of H 80/62 used in the present experiments was 180  $\mu\text{g/kg}$ .

### II Experiments with isolated rabbit lacrimal glands

Rabbits of either sex were killed by stunning and bleeding and the lacrimal glands were rapidly excised through the caudal supraorbital incision and freed from its capsula. The lacrimal gland was mounted in a small organ bath and suspended in a Krebs-Henseleit solution containing (mmol/l): NaCl 119.0, MgCl<sub>2</sub>

1.25  $\text{NaHCO}_3$  15.4  $\text{KH}_2\text{PO}_4$  1.2 glucose 5.5 and caseine sulphate 0.03 solution was kept at  $37^\circ\text{C}$  and aerated with a mixture of  $\text{O}_2$  (95%) and  $\text{CO}_2$  (5%)

The preparation was incubated with  $10\text{ }\mu\text{mol/l}$  of [ $^3\text{H}$ ]choline (methylcholinechloride  $10.0\text{ }\mu\text{Ci/ml}$ ) for 60 min and during that period it was continuously given electrical field stimulation with a supramaximal voltage 1 msec and 1 Hz from two transversally mounted platinum electrodes. After incubation in [ $^3\text{H}$ ]choline the preparation was washed by superfusion with Krebs solution for 30 min. Then 4 ml samples of the superfusate were taken every 5 min during the whole experiment for analysis of [ $^3\text{H}$ ]choline and [ $^3\text{H}$ ]acetylcholine according to Wikberg (1971). The [ $^3\text{H}$ ]acetylcholine was also purified by ion exchange chromatography according to a double isotope technique described by Wikberg (1979). At the end of each experiment the radioactivity of the whole lacrimal gland was determined by dissolving it in 1 ml scintivene<sup>®</sup>. Then 10 ml of a scintillation fluid containing 50 mg PPO (diphenyl oxazole) and 3 mg POPOP (2,2',5'-phenylene bis (5-phenyloxazole)) in toluene was added and the radioactivity was measured in a Packard Tri Carb scintillation counter. The counting efficiency was determined by an internal standard and varied between 30–35 per cent for the different samples. In the present experiments the release of [ $^3\text{H}$ ]choline and [ $^3\text{H}$ ]acetylcholine into the superfusate were calculated as per cent of the total radioactivity calculated to be present in the tissue before the first fraction was collected (Wikberg 1971). In order to study the effect of adrenergic  $\beta$  stimulation on the release of acetylcholine isoprenaline was added to the superfusing Krebs solution both electrically stimulated and to unstimulated tissues.

Mean values, standard error of mean values and Student's *t* test were calculated on a computer using standard formulas.

## Results

### Experiments with conscious rabbits

**Effects of isoprenaline on basal tear flow** In some preliminary experiments it was found that isoprenaline  $0.3\text{ }\mu\text{g/kg/min}$  infused intravenously in  $0.2\text{ ml}$  of saline/min increased the tear flow by maximally  $25 \pm 3$  per cent ( $N = 5$ ). The increase in tear flow was fairly stable for at least an infusion period of 20 min. Isoprenaline  $0.3\text{ }\mu\text{g/kg min}$  increased the heart rate by maximally  $115 \pm 10$  beats/min ( $N = 4$ ) as measured from the ECG. The effects of isoprenaline  $0.3$

$\mu\text{g/kg/min}$  were submaximal as isoprenaline  $1.0 \mu\text{g/kg/min}$  caused an increase in tear flow of maximally  $51 \pm 4$  per cent ( $N = 4$ ) and increased the heart rate by maximally  $172 \pm 16$  beats/min ( $N = 4$ ) (Fig. 1).

**Effects of  $\beta$  blocking agents on basal tear flow.** Neither propranolol (Inderal) in doses up to  $6.0 \text{ mg/kg}$  nor metoprolol (Seloken® Iopressor®) in doses up to  $30.0 \text{ mg/kg}$  had any significant effects on basal tear flow (Table 1).

**Effects of  $\beta$  blocking agents on isoprenaline induced increase of tear flow.** Both propranolol and metoprolol decreased the isoprenaline induced increase of tear flow in a dose dependent manner (Figs 2 and 3).  $\text{ED}_{50}$  for the propranolol induced decrease of tear flow was approximately  $1.0 \text{ mg/kg}$ . The corresponding value for the  $\beta_1$  selective blocker metoprolol was approximately  $5.0 \text{ mg/kg}$  (Fig. 3).

**Effects of H 80 62 on basal tear flow.** The basal tear flow was increased by H 80 62. In dose finding experiments it was found that H 80 62 given intravenously in the dose  $60 \mu\text{g/kg/min}$  for 3 min increased the basal tear flow with maximally  $37 \pm 7$  per cent ( $N = 6$ ) i.e. slightly more than a  $10 \mu\text{g/kg/min}$  infusion of isoprenaline  $0.3 \mu\text{g/kg/min}$ . The dose of H 80 62 presently used was a submaximal one as after H 80 62  $300 \mu\text{g/kg/min}$  for 3 min the

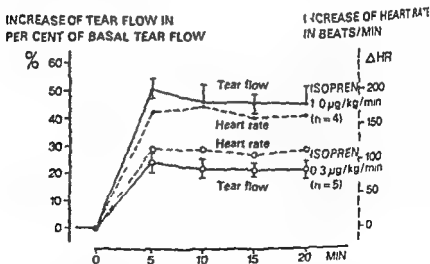


Fig. 1

Effects of isoprenaline on basal tear flow and heart rate when given intravenously for 2 min in the doses  $0.3$  and  $1.0 \text{ mg/kg/min}$  to conscious rabbits. For heart rate standard errors of the means are not shown for the sake of simplicity the standard errors were consistently less than 10 per cent of the mean values shown in the figure.

Table 1

Effects of  $\beta$  blockers on basal tear flow. The basal tear flow 3 min before intravenous administration of saline or a  $\beta$  blocker was called 100 per cent. No statistically significant changes in the basal tear flow were caused by the  $\beta$  blockers ( $P > 0.05$ ).

$\beta$ blocker	Dose (mg/kg)	N	Tear flow (%) at two different times after $\beta$ blockade (mean $\pm$ SEM)	
			3	15
NaCl 0.9%	-	15	100 $\pm$ 4	97 $\pm$ 3
Propranolol	10	6	107 $\pm$ 7	110 $\pm$ 7
"	30	4	99 $\pm$ 8	97 $\pm$ 11
"	60	4	104 $\pm$ 8	99 $\pm$ 8
Metoprolol	30	9	99 $\pm$ 1	100 $\pm$ 4
"	100	8	100 $\pm$ 1	100 $\pm$ 6
"	300	4	100 $\pm$ 5	101 $\pm$ 5

CHANGE IN TEAR FLOW IN PER CENT OF BASAL TEAR FLOW

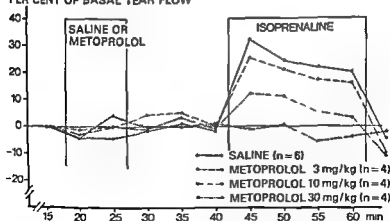


Fig. 2

Effects of metoprolol 3, 10 and 30 mg/kg on isoprenaline induced increase of tear flow. The SEM values are not shown for simplicity reasons. During the infusion of isoprenaline SEM was less than 5 per cent of the basal tear flow.

# INHIBITION OF ISOPRENALINE INDUCED TEAR FLOW

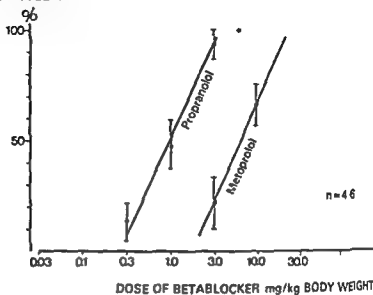


Fig 3

Inhibitory effects of metoprolol and propranolol on isoprenaline induced increase of tear flow

## INCREASE OF TEAR FLOW IN PER CENT OF BASAL TEAR FLOW

## INCREASE OF HEART RATE IN BEATS/MIN

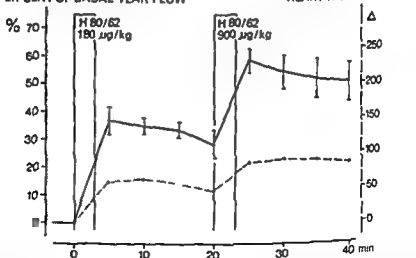


Fig 4

Effects of H 80 62 on basal tear flow ( $N=3$ ) and heart rate ( $N=3$ ) when given intravenously in the doses 180 + 900 mg/kg. The SEM values for heart rate were consistently less than 10 per cent of the mean values shown in the figure

flow was increased maximally by  $58 \pm 4$  per cent ( $N=6$ ). The maximal rise in heart rate was  $62 \pm 4$  beats/min after H 80/62 in the lower dose (3) and  $92 \pm 4$  beats/min after the higher dose ( $N=3$ ) (Fig. 4).

**Effects of metoprolol on H 80/62 induced increase in tear flow** The H 80/62 induced increase of the tear flow was dose dependently decreased by metoprolol 0.3–10 mg/kg. The  $ED_{50}$  value for metoprolol in these experiments was approximately 1.0 mg/kg (Figs. 5 and 6).

#### Experiments with isolated rabbit lacrimal glands

The spontaneous release of [ $^3$ H]choline from isolated lacrimal glands was approximately 10 times higher than the release of [ $^3$ H]acetylcholine. Electrical stimulation with 4 Hz 1 ms and with 10 Hz 1 ms induced a four fold and a nine fold increase in the release of [ $^3$ H]acetylcholine (Figs. 7 and 8).

Isoprenaline ( $3 \times 10^{-5}$  mol/l) had no significant effect on the [ $^3$ H]acetylcholine release neither during unstimulated conditions (Fig. 7) nor during continuous stimulation at 4 Hz (Fig. 8). The release of [ $^3$ H]choline was however in both instances increased by isoprenaline.

#### CHANGE IN TEAR FLOW IN PER CENT OF BASAL TEAR FLOW

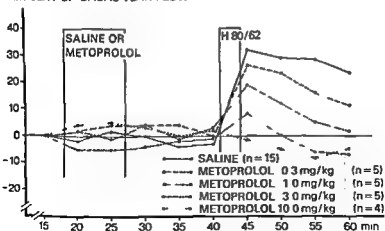


Fig. 5

Effects of metoprolol 0.3, 1.0, 3.0 and 10.0 mg/kg on H 80/62 induced increase of tear flow. After the infusions of H 80/62 the SEM values were consistently less than 5 per cent of the basal tear flow.

# INHIBITION OF H 80/62 INDUCED TEAR FLOW

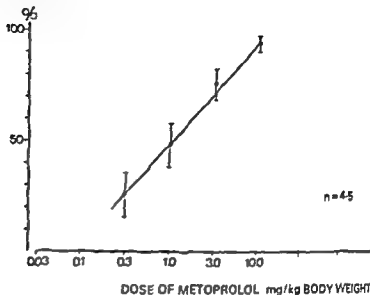


Fig 6

Inhibitory effect of metoprolol on H 80/62 induced increase of tear flow

## RELEASE H PER CENT OF TISSUE ACTIVITY

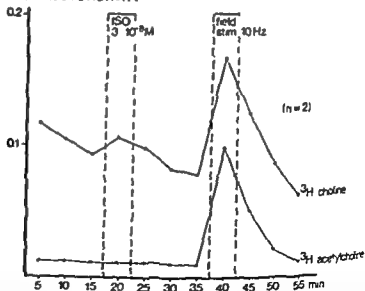


Fig 7

Release of [ $^3\text{H}$ ]acetylcholine and [ $^3\text{H}$ ]choline from isolated rabbit lacrimal gland. The preparations were stimulated first by isoprenaline ( $3 \times 10^{-8} \text{ M}$ ) for 5 min and then electrically (10 Hz 10 msec) for 5 min as indicated in the figure.

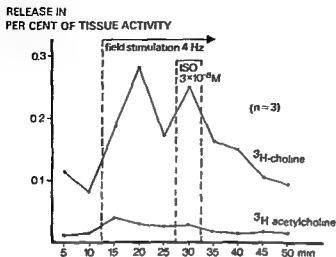


Fig 5

Release of [<sup>3</sup>H]acetylcholine and [<sup>3</sup>H]choline from isolated rabbit lacrimal glands. These preparations were given a continuous electric field stimulation (4 Hz 10 msec) and isoprenaline was added to the bath solution for 5 min as indicated in the figure.

## Discussion

The main nervous supply to the lacrimal gland is of parasympathetic origin (Semtschenko 18/2). The existence of a sympathetic nerve supply to the lacrimal gland is however under discussion. Thus from electrophysiological experiments Botelho et al (1966) concluded that if sympathetic nerve impulses influence the secretory activity of the lacrimal gland these impulses do not reach the gland via the lacrimal nerve. After cannulation of the excretory duct of the inferior lacrimal gland an increase in tear flow has however been recorded after parenteral administration of noradrenaline to anaesthetized rabbits (Goldstein et al 1967). In addition to these findings Botelho et al (19/3) have demonstrated that not only noradrenaline but also isoprenaline may increase the tear flow in anaesthetized rabbits isoprenaline being even more effective than noradrenaline. Botelho and her co workers also showed that the increase in tear flow produced by these drugs was abolished by the adrenergic blocking compound sotalol. They concluded that in the rabbit lacrimal gland there are  $\beta$  adrenergic receptors which are most likely located in the secretory cells. The findings of Botelho et al (19/3) have recently been verified by Bissier et al (19/6). They found that an isoprenaline induced increase in the tear flow in rabbits was decreased by propranolol.



In the present investigation we found a dose dependent inhibition of isoprenaline induced increase in tear flow by propranolol as well as the selective antagonist metoprolol (Åblad et al 1973). The  $ED_{50}$  values for inhibition was 1.0 and 5.0 mg/kg respectively for propranolol and metoprolol (Fig. 3). That relation between the two  $ED_{50}$  values is approximately the same as the relationship between their inhibitory activity against isoprenaline induced cardiac effects (Åblad et al 1973). This indicates that the adrenergic  $\beta$  receptors involved in lacrimal secretion were of the  $\beta_1$  type. In an additional series of experiments it was found that the tear flow was increased by the  $\beta_1$  selective agonist H 80 62 (Carlsson et al 1977) and this increase was completely blocked by the  $\beta_1$  selective antagonist metoprolol. We therefore conclude that the  $\beta$  receptors involved in the regulation of tear flow are of the  $\beta_1$  type.

The localization of these  $\beta_1$  receptors is still unclear. Theoretically they can be located either at the lacrimal secretory cells and directly influence the activities of the cells or they may be located at the cholinergic neurons and influence the release of acetylcholine. In our *in vitro* experiments isoprenaline did not change the amount of acetylcholine released during electrical stimulation. Therefore we are inclined to dismiss prejunctional sites on the cholinergic nerve endings as possible locations for the  $\beta_1$  receptors although it was an interesting finding that [<sup>3</sup>H]choline in these experiments was liberated when isoprenaline was added to the bath fluid. Thus the most probable site for the tear flow regulating  $\beta_1$  receptors in the intact animal are the lacrimal glandular cells. There may also be vasodilating  $\beta_1$  receptors in the lacrimal gland tissue as demonstrated for canine adipose tissue by Belfrage (1976).

It may be concluded from the present investigation that the decrease in tear flow induced by adrenergic  $\beta$  blockers in this study was a pharmacological effect depending on an inhibition of  $\beta_1$  receptors and not an unspecific toxic effect elicited by the  $\beta$  blocking agent.

### Acknowledgment

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# THE EFFECT OF METOPROLOL ON INTRA OCULAR PRESSURE IN GLAUCOMA

## A Pilot Study

BY

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and LENA ÖHMAN

Eleven patients with previously untreated glaucoma of one eye were given metoprolol 50 mg  $\times$  3 for one day. Intraocular pressure (IOP) and heart rate were significantly reduced during treatment and increased after withdrawal of the drug. In the eyes with glaucoma the mean IOP was reduced from 30.1 to 20.6 mmHg during treatment. Systolic and diastolic blood pressures were also reduced during treatment but stayed at the same level after withdrawal of the drug. It is concluded that at least part of the reductions observed for IOP and heart rate are drug induced while the effect on blood pressure is mainly induced by factors other than the drug. The effect on IOP is considered large enough to be of clinical interest.

**Key words:** beta adrenergic blockers metoprolol - glaucoma - intraocular pressure

It has previously been shown that beta adrenergic blockers reduce intraocular pressure (IOP) whether they selectively block  $\beta_1$  receptors (Vale & Phillips 1973; Wettrell & Pandolfi 1977) or both  $\beta_1$  and  $\beta_2$  (Phillips et al 1961; Zerman & Krusman 1977). Metoprolol (Seloken  $\text{\textcircled{S}}$ ) is a selective  $\beta_1$  blocker without intrinsic activity and with only weak membrane stabilising properties (Ablad et al 1973) and the present study was undertaken to determine whether IOP is reduced by metoprolol. The effect on heart rate and blood pressure was also determined.

## Material and Methods

Patients were chosen according to the following criteria

**Inclusion criteria** 1) Previously untreated open angle glaucoma with or without lens exfoliations. The diagnosis was based on the existence of glaucoma with visual field defects or uniocular IOP above 30 mmHg on repeated determinations. 2) IOP 30 mmHg or more measured at least on two occasions during the first 24 h at the hospital. IOP was measured every third hour except between 12 p.m. and 6 a.m.

**Exclusion criteria** 1) Atrioventricular block seen on ECG. 2) IOP more than 40 mmHg. 3) Arterial blood pressure above 180 mmHg systolic and/or 95 mmHg diastolic. 4) Previous treatment with beta adrenergic blockers. 5) Diabetes mellitus. 6) bronchial asthma or manifest cardiac compensation.

**Description of the material** A total of 11 patients consented to enter the study. There were 5 men and 6 women, aged 51 to 82 years. IOP was determined in both eyes with one exception (one eye was excluded due to chronic iridocyclitis). The main object of the present study was to obtain information about the effect of metoprolol on glaucomatous eyes. Eleven eyes, one from each patient, suited the inclusion criteria and were treated as one group in the statistical calculations. This group will in the following be referred to as eye 1. For the remaining 10 eyes the highest IOP observed was 27 mmHg. Statistical calculations for this group will be presented as eye 2.

The study lasted three days, not including the first day at the hospital which was used to determine the diurnal pressure curve, the ECG and to inform the patient of the study.

**Day 1** IOP was determined at 8 a.m., 2 p.m. and 8 p.m. with a Goldmann applanation tonometer. IOP was determined by one of the authors. Heart rate and blood pressure were determined at 8 a.m. and 8 p.m. by a nurse and at 2 p.m. by one of the authors. Heart rate and blood pressure were determined after at least 5 min rest.

**Day 2** 50 mg metoprolol (Seloken®) was given orally at 6 a.m., 12 and 8 p.m. Heart rate and blood pressure were determined as on day 1.

**Day 3** No metoprolol was given and measurements were made as on days 1 and 2 in all patients except one where no measurements were made at 8 p.m.

During this time no other glaucoma therapy was given. After the study the patients were questioned about side effects.

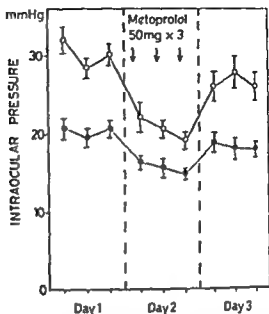


Fig. 1

The IOI mean  $\pm$  SEM in the glaucomatous eyes (eye 1 - open circle) and the healthy eyes (eye 2 - closed circle) at 8 a.m., 2 p.m. and 8 p.m. for three days. Metoprolol 50 mg was given 2 h before measurements of IOI on day 2 (arrows).

## Results

Fig. 1 shows the results of the IOP determinations at 8 a.m., 2 p.m. and 8 p.m. for the three days of the study. Mean values and SEM are given. Fig. 2 shows the corresponding values for heart rate and blood pressures.

The mean values observed at 8 a.m., 2 p.m. and 8 p.m. did not differ significantly from each other on either day or for either parameter studied (Figs 1 and 2). The mean pre-treatment values calculated from the average of the measurements on day 1 were IOP eye 1  $30.1 \pm 1.2$  mmHg, eye 2  $20.5 \pm 1.1$  mmHg, systolic blood pressure  $154 \pm 3$  mmHg, diastolic blood pressure  $82 \pm 2$  mmHg and heart rate  $74 \pm 2$  (Mean  $\pm$  SEM). Averaged values for days 1 and 3 were used to calculate the differences observed between the various days. Table 1 presents such differences based on paired data. The difference (day 1 - day 2) shows the change observed when the drug was given and the difference (day 3 - day 2) the degree of recovery when the drug was withdrawn. IOPs in both eyes and heart rate were reduced on day 2 and showed an incomplete recovery during day 3. All differences were statistically significant. Systolic

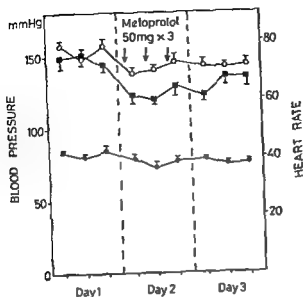


Fig 1

Heart rate and blood pressures Times for measurement and administration of metoprolol as in Fig 1 Mean and 1 SEM are presented

○ Systolic blood pressure  
● Diastolic blood pressure  
■ Heart rate

Table 1

Differences based on paired data for the mean values of day 1 2 and 3 respectively  
mean  $\pm$  SEM (n=number of patients) and statistical significance (Student's *t* test)  
n.s. not significant

Days for which the difference was calculated	IOP Eye 1 mmHg (n=11)	IOP Eye 2 mmHg (n=10)	Heart rate (n=11)	Syst BP mmHg (n=11)	Diast BP mmHg (n=11)
Day 1 - Day 2	9.0 $\pm$ 1.1 <i>P</i> < 0.001	4.1 $\pm$ 0.9 <i>P</i> < 0.001	12.0 $\pm$ 1.3 <i>P</i> < 0.001	12.3 $\pm$ 3.4 <i>P</i> < 0.01	6.5 $\pm$ 1.8 <i>P</i> < 0.005
Day 3 - Day 2	6.3 $\pm$ 1.1 <i>P</i> < 0.001	2.7 $\pm$ 0.6 <i>P</i> < 0.005	3.6 $\pm$ 1.3 <i>P</i> < 0.025	1.2 $\pm$ 2.2 n.s.	0.2 $\pm$ 2.2 n.s.

and diastolic blood pressures also showed a significant reduction on day 2, no recovery was observed on day 3.

One patient complained of slight fatigue during day 2 but otherwise no effects were observed.

## Discussion

The aim of this pilot study was to examine the possibility that metoprolol has an IOP lowering effect large enough to be of clinical interest. The dose of 150 mg is comparable to 160 mg propranolol regarding the effect on heart and blood pressure (Johnsson et al 1975) and it was chosen since 80 mg propranolol twice daily is known to have a marked possibly maximal effect on IOP (Wettrell & Pandolfi 1976, Borthne 1976). IOP was determined 4 h after administration of the drug. At this time the effect of the drug can be expected to be almost maximal.

In the present study significant reductions were observed for all parameters observed on day 2 but the degree of recovery on day 3 varied (Table 1). A significant recovery on day 3 indicated that at least part of the observed effect on day 2 is drug induced while no recovery may be explained by the possibility that the effect observed on day 2 is a placebo effect or that the drug effect remains. Since the time for a 50% reduction of the maximum effect of 100 mg metoprolol on exercise heart rate and blood pressure is about 8 h (Regårdh 1975) it is possible that some drug effect remains at 8 a.m. on day 3 but is likely at 5 p.m. Table 1 presents only the differences based on the mean values for the different days but as seen in Figs 1 and 2 no significant difference in recovery was observed between 8 a.m. and 8 p.m. on day 3 with the possible exception of heart rate. Thus it is possible that for heart rate part of the complete recovery on day 3 is due to remaining drug effect at 8 a.m. resulting in a disproportionate low mean value. In fact the difference in heart rate between day 1 and 3 at 5 p.m. is not significant ( $4.6 \pm 2.4$  0.05 <  $P$  < 0.01 Student's  $t$  test paired data). It may then be concluded that in the present study metoprolol reduced IOP and heart rate while the blood pressure reduction observed on day 2 and 3 was mainly induced by factors other than the drug, e.g. decreased anxiety in these patients with a recently diagnosed glaucoma. The conclusion that the observed blood pressure reduction was not drug induced may be surprising considering the known blood pressure reducing effects of various beta adrenergic blockers including metoprolol (Berens 1976). However in the present study blood pressure was determined at rest in patients without known arterial hypertension and after only one day's treatment.

administration The exact mechanism by which beta adrenergic blockers reduce blood pressure is not known but it is believed to be secondary to a reduction in cardiac output and renin release (Simpson 1974) and in persons with normal blood pressure it is most marked during exercise and may have no hypotensive effect in rest (Schroder & Werko 1964) An initial blood pressure reduction may not be observed even in patients with arterial hypertension since the reduced cardiac output is at first compensated by an increased peripheral resistance (Hansson et al 1974) which will gradually return towards the initial value or even less Still there is no reason to doubt that long term oral treatment of metoprolol in patients with glaucoma will cause a true reduction of blood pressure and with metoprolol as with other beta adrenergic blockers topical application of eye drops would be preferable since a reduced blood pressure may diminish the benefit of a reduced IOP

The effect on IOP in the present study is considered large enough to be of clinical interest and it is of the same order as found for propranolol in a similar study (Borthne 1976) However until dose response relationship and duration have been determined meaningful comparisons cannot be made with other beta adrenergic blockers Since the completion of the present study Ros et al (1978) have reported the effect of metoprolol eye drops These investigators found a maximum effect for 1 per cent metoprolol with a duration of at least 8 h but no similar study on oral administration of metoprolol is known to us The results of the present study however encourage further studies on the effect of metoprolol on normal and increased IOP

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## CORNEAL ASTIGMATISM AFTER CATARACT EXTRACTION

### A Comparison of Corneal and Corneoscleral Incisions

BY

JOHN THYGESEN PER REERSTED HANS FLEDELIUS  
and LEIF CORYDON

Corneal astigmatism after cataract surgery by way of corneal incision (C n=62) and corneo scleral incision (CS n=61) was followed for six months. Corneal incisions were closed by continuous nylon 10-0 (-25 loops median 14). Corneo scleral incisions were sutured with single knots (2-10 median value 5).

Keratometric results in the C and CS groups are compared. Concerning the degree of astigmatism pre operative median values were 0.5 and 0.7 D respectively. After one week they were 4.5 and 3.0 D, after two weeks 3.3 and 3.0 D, after four months 3.0 and 2.0 D, after six months (final status) 1.5 and 1.0 D respectively. The differences between C and CS were not significant. For both astigmatism after cataract surgery did not quite return to pre operative levels.

Concerning the axis (weaker meridian) of corneal astigmatism the C cases retained their pre operative distribution while the CS cases showed the classical shift towards *against rule* astigmatism.

Final corrected visual acuity was of the same order in the C and CS group.

Due to frequent shifts also of the axis it is recommended by early (preliminary) glass prescription not to correct the astigmatism but to give only the best spherical correction.

**Key words:** cataract extraction - corneal incision - corneo scleral incision - corneal astigmatism

The material was presented at the Danish Ophthalmological Society April 15 1978  
received September 22 1978

During the last years a corneal incision (under the microscope) has found increasing use in our department in cataract surgery. Inevitably discussion has arisen concerning the advantages and drawbacks as compared with the routine method in use until then, namely an ab externo corneo scleral incision with a limbus based conjunctival flap. It thus appeared that corneal astigmatism was often high after a corneal incision at least initially and apparently the patient had to wait longer for definitive glass prescription.

To clarify these points a consecutive series of cataract extraction by the two methods was followed for six months and the results were compared. The degree of and the change in post operative corneal astigmatism is the subject of the present publication.

## Materials and Methods

123 consecutive cataract extractions from May to November 1976 were included in the study.

Excluded were only 1) cases in which keratometry was not possible due to pre-existing corneal pathology and 2) patients where geography was inhibitory to follow up (Greenland Faroe Islands remote parts of Denmark).

The bulk of the material was made up of senile and presenile cataracts but there were also some complicated cataracts (uveitis excessive myopia heterochromia). Cases with complications during surgery were not excluded.

Age of patients ranged from 33-91 years in the corneal and 54-80 years in the corneo scleral group. There were 63 female and 60 male eyes 67 right and 56 left eyes.

62 patients had a corneal incision and a continuous nylon 10-0 suture except for six cases with single sutures (virgin silk vicryl dextron). The number of loops was 1-20 median value 14.

61 patients had a corneo scleral incision under a limbus based conjunctival flap. Single sutures were used in a number from 2 to 10 median value 5. Virgin silk was used in 33 vicryl in 23 and collagen in 6.

The incision was made ab externo in two steps and angled first with knife (a) and then with a razor blade perpendicular to surface to a depth of  $\frac{2}{3}$  of the cornea and then with scissors. All cases but six were operated on under the microscope.

The operations were performed - usually in local anaesthesia - by members of the staff: a) Chief surgeons 51 cases (39 corneal 19 corneo scleral) and b) Residents 66 cases (34 corneal 42 corneo scleral).

There was a slight load of heavy cases in the corneal group (because in general a corneal incision was chosen in cases where complications were expected). Accordingly vitreous loss occurred more often in this group (1/62) than in the corneo-scleral group (2/61). A transitory rise in IOP (above 24 mmHg) was found in four and one eye respectively. There was one case of flat chamber after surgery (corneo-scleral incision) and one case of secondary haemorrhage (corneal incision). Clip lenses were implanted in seven corneal and in two corneo scleral cases.

## Examination scheme

$\Delta K$  (corneal astigmatism in dioptres) the axis (weaker corneal meridian in degrees) and corneal power (average between weaker and stronger meridian in dioptres) were measured by Javal Schiotz *keratometry* 1) pre operatively 2) one week after 3) two weeks after 4) four months after (where the nylon suture was removed in corneal cases) and 5) six months after surgery (defined as final status). On the last occasion *refraction* (spherical equivalent in dioptres) and corrected visual acuity were also recorded.

## Statistical methods

Data were put on punch cards. Standard computer programs disclosed that *non parametric* statistics were to be preferred (due to the distribution of the two main parameters the keratometric  $\Delta K$  value and the orientation of the axis). *P* values are given when significant ( $P < 0.05$ ).

## Results

At the post operative follow up examinations there were a few fall outs and a fact that is reflected in the varying numbers of eyes shown in Fig. 8.

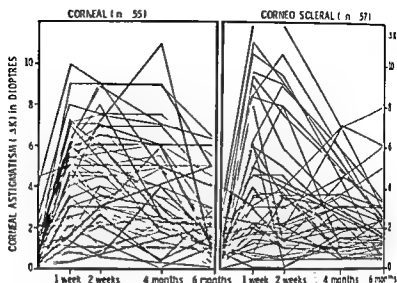
In the following the designations C and CS will be used for the corneal and corneo scleral group respectively.

Table I shows the corneal astigmatism before surgery and on the four post operative occasions. Mean values and standard deviations are shown to allow comparison with earlier relevant studies but the median value is preferred as a more true indicator. Prior to surgery there is no difference between the C

		Pre op	1 week	2 weeks	4 months	6 months
$\Delta K$ in Dioptres MEAN VAL $\pm$ SD	Corneal	<u>10</u> $\pm 10$	<u>42</u> $\pm 23$	<u>40</u> $\pm 24$	<u>37</u> $\pm 24$	<u>22</u> $\pm 17$
	Corneo-scleral	<u>09</u> $\pm 08$	<u>40</u> $\pm 29$	<u>36</u> $\pm 32$	<u>23</u> $\pm 16$	<u>20</u> $\pm 16$
$\Delta K$ in Dioptres MEDIAN VAL (range)	Corneal	<u>05</u> (0 45)	<u>45</u> (05 10)	<u>33</u> (05 9)	<u>30</u> (0 11)	<u>15</u> (0 65)
	Corneo scleral	<u>07</u> (0 40)	<u>30</u> (0 12)	<u>30</u> (0 18)	<u>20</u> (05 7)	<u>17</u> (0 80)

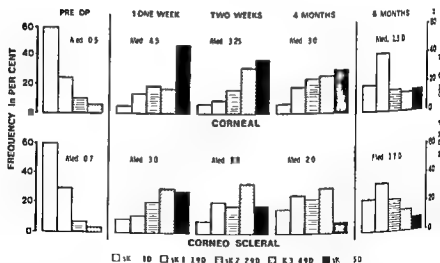
Table I

Corneal astigmatism  $\Delta K$  before and after cataract extraction on four post operative occasions. Mean values and SD (top) median values and range (bottom) Corneal and corneo scleral cases.



*Fig 1*

Corneal astigmatism before and after cataract extraction recordings of angle  $\alpha$   
Corneal group left corneo scleral group right



*Fig 2*

Corneal astigmatism prior to surgery (left) and on four occasions after cataract extraction Percentage distribution on five  $\Delta K$  classes Corneal cases (top) corneo scleral cases (bottom)

1 CS group and the same applies to the final  $\Delta K$  value. The four months post-surgery however leaves the impression that CS cases achieve their final  $\Delta K$  value somewhat earlier than the C cases.

The same is evident from Figs 1 and 2 which show longitudinal recordings in single cases (Fig 1) and percentage distributions (Fig 2). In Fig 1 there is in the C group a rather high  $\Delta K$  plateau until suture removal (after four months) while normalization occurs earlier among those with CS. According to the darker (less favourable high  $\Delta K$  classes) columns of Fig 2 show an abrupt increase in relation to surgery followed by a gradual return to a distribution which comes closer to the status before surgery.

Assessed by  $\chi^2$  tests there is no significant difference between C and CS on five occasions i.e. by vertical comparisons of the distributions shown in Fig 2. By horizontal assessment - within the C and CS group respectively - there is a highly significant difference between the  $\Delta K$  distribution before surgery and at the final status ( $P < 0.001$ ) and this applies to both groups. Corneal astigmatism thus does not return to preoperative levels.

The axial orientation of corneal astigmatism (position of weaker meridian) is recorded on the keratometer and transferred to punch card in its relevant degree box (see Fig 3). Astigmatism with the rule comprised cases with the axis between  $0-20^\circ$  and  $160-180^\circ$ . Against the rule broadly com-

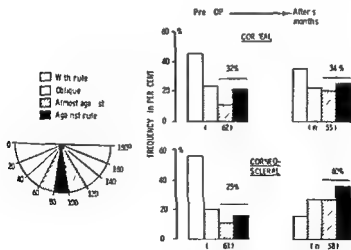


Fig 3

Axial orientation of corneal astigmatism prior to surgery for cataract (left) and 6 months after surgery (right) with corneal cases (top) and corneo scleral cases (bottom). Percentage distributions.

prised the zone 60–120° but "against" in a narrow sense ( $90 \pm 10^\circ$ ) is represented and specified with black in Fig. 3. Oblique astigmatism occupies the remaining  $2 \times 10^\circ$ .

Before surgery about 50% were with rule and there was no difference between C and CS groups. At the final status after six months the change in axial orientation (distributions of Fig. 3) was not significant within the group which maintained a 1/2 share of "against rule" astigmatism. Conversely there was a significant shift ( $P < 0.001$ ) in the CS group with a marked reduction in "with rule" astigmatism and a marked rise in the "against rule" fraction (from 25 to 60%). The final C and CS status differ accordingly but only at a 0.05 level.

The material enables us to give an answer to a practical question: "Can glasses be prescribed early?" Because if significant changes occur in only a minor fraction the major part of patients might have the benefit of an early full prescription. This however did not hold for any of the groups as only single cases were followed (in contrast to the summary distributions of Fig. 1). As concerns *ΔK*, about 50% had changes exceeding 2 dioptres between 14 days after surgery and six months. Further, about fifty per cent showed an *Δα* shift exceeding 20 degrees in the same period.

The above results are considered the main object of the present study. In addition a summary mention will be given of some other data.

*Corneal power* (range 39.3–48.5 D) did not change much in relation to surgery. After exclusion of one eye with a presumed faulty recording (of 33.5 in the C group) the C cases had the mean value 43.28 D ( $\pm 1.66$ ) before surgery and 43.13 ( $\pm 1.99$ ) after six months. The corresponding CS values were 43.06 ( $\pm 1.66$ ) and 43.09 D ( $\pm 1.89$ ).

Final corrected *visual acuity* was good in both groups with values  $\geq 60/84$  84%. Visual acuity  $\leq 6/60$  (pre-existing maculopathy: 2 cases; aphakic retinal detachment: 1 case) occurred in one eye with C and in two with CS.

There was no correlation between astigmatic error (*ΔK*) and final corrected visual acuity.

After exclusion of the nine eyes with clip lenses the final refraction (spherical equivalents) of C cases showed a significant shift ( $P < 0.01$ ) towards lower values when compared with the CS group. This is reflected also in the mean values of the two groups being 11.1 D ( $\pm 3.3$ ) and 12.9 D ( $\pm 1.9$ ) respectively. As deducted from the aphakic refractive values the C group thus entailed eight eyes that had high myopia prior to surgery against only one eye in the CS group.

The final post-operative astigmatism showed no correlation (Spearman's rank) to pre-operative corneal astigmatism, age of patient, number of surgical loops or to visual acuity (vide supra).

## DISCUSSION

Advances in surgery (microsurgical techniques) and pharmacology (access to antibiotics and corticosteroids) have by and large eliminated the formerly dreaded severe complications of cataract extraction. Therefore with the words Troutman (1970) we can now begin to worry about the previously neglected details. One area which has been ignored is the optical distortion of the cornea randomly induced by current cataract incision and closure over which chance still has the best control.

This degree of corneal distortion is the sole subject of the present publication which does *not* deal with other aspects of cataract surgery. Let it only be stated that a corneal incision has been used because in general this is regarded as being pretty safe (Corydon 1976, Corydon & Mackensen 1978) and especially so in eyes with expected complications to surgery (a fact that is also reflected by the greater share of heavy cases in the corneal group of the present material).

The ideal set up for a comparison of the two cataract surgery incisions would be as follows. The same surgeon should perform the surgery in all cases and with equal skill and experience as regards the two incisions to be compared. Allocation to the two techniques should be strictly at random. Regard should be taken to type of knife and scissors, to suture material and technique as well as to the age of the patient, sex, type of cataract, eye size etc.

Such demands are hard to fulfil in a clinical series. This is apparent from the present material as well as those of earlier reports on the subject. We managed to keep the unity of time and place but a considerable number of surgeons were included, experienced as well as residents and the techniques of wound closure varied. Corneal incisions were usually closed by a continuous nylon suture while corneo-scleral incisions were sutured by single sutures in a varying number and with different materials.

The present approach is therefore primarily practical, clinical and does not eliminate multifactorial hazards but we still consider a comparison of the two methods as being justified. This may be supported by the fact that the corneal astigmatism after surgery by residents equalled the results gained by chief surgeons. A similar experience was reported earlier by Bedrossian *et al.* (1969).

From our results we may advance that the *final* degree of corneal astigmatism showed no relation to the type of incision. Further, our 12 months values equalled those of a quite recent German study (Steinbach & Gerhard 1978) with a set up quite similar to the present one. In both studies however, nor



malization seemed somewhat retarded in the group with corneal incision due to the late removal of the non absorbable nylon suture

Concerning the axis of astigmatism there is however a remarkable difference between the two studies Steinbach & Gerhardt (1978) found - for both incisions - a shift in axis from with the rule towards against rule (vertical meridian vertically) This is the classical experience gained since the Graefes incision was introduced but it does not accord with more recent reports that emphasise a more "natural tendency" (with rule) after corneal incision with meticulous wound closure under the microscope In our material this was not evident We found a significant difference between the classical against rule corneo scleral cases and those with a corneal incision The latter largely kept their original distribution of axial directions (Fig 3)

The impact of the present study is that the end results concerning corneal astigmatism after the two different incisions were pretty similar however with the exception mentioned regarding the axis This had but little influence on the functional end result (which is corrected visual acuity) We think that Steinbach & Gerhardt (1978) - that both methods are from an astigmatism point of view equally suited for cataract surgery And further modern microsurgical advances have now modified one of the classical dogmas about cataract surgery that astigmatism will increase the more corneal (nearer to vertex) incision is located (Lundsgaard 1925)

It is tempting finally to quote another countryman Edmund who at the same meeting in 1925 stated that "Nature has a peculiar ability to let all external actions altered anatomy return to its original norm"

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In addition, reference is made to essential contributions in a recent publication *Recent Concepts in Cataract Surgery* (Emery J M and Paton D Eds 1976 C V Mosby) which came to our knowledge after the present paper was finished

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## EARLY POSTOPERATIVE CHANGES IN GRAFT THICKNESS AFTER PENETRATING KERATOPLASTY

Influence of Host Corneal Disorder on Time Course

BY

THORKILD BRAMSEN and NIELS EHLERS

In 12 patients the thickness of the corneal graft was followed with frequent measurements during the first 14 days after operation. Three different graft thickness time courses were observed. Patients with keratic stromal dystrophy and corrosion or mechanical lesion showed a secondary rise in graft thickness on the 6th postoperative day while patients with keratoconus and those treated with tranexamic acid showed no rise on the 6th day. Patients with Fuchs' dystrophy differed from the other groups in not reaching the maximal thickness until the 3rd postoperative day.

The possible correlation of these three time courses with changes in the fibrinolytic system is discussed.

**Key words:** transplantation - graft thickness - pachometry - fibrinolysis - keratoconus - Fuchs' dystrophy - keratitis

The regulation of the corneal thickness after an operation or after an ocular inflammation is probably influenced by the same mechanisms which maintain the normal thickness. It may therefore be of interest to follow the time course of this normalization by frequent measurements of the central corneal thickness (CCT) after ocular surgery. The most marked changes in CCT occur shortly after the operation and often the thickness is normal within a few weeks. Changes in thickness may reflect a changing activity of the mechanisms con-

ling the thickness Various time courses of CCT after surgery in cases of  
ferent ocular disorders may give a clue to the pathogenesis of these con-  
itions

Investigations concerning the influence of the antifibrinolytic drug tranexamic acid (Cyclokapron®) on the CCT after intraocular operations (Bram et al 1978 Bramsen 1978) and a study of the effect of this drug on CCT cases of Fuchs endothelial dystrophy (Bramsen & Ehlers 1977) have shown that tranexamic acid has a thickness reducing effect in pathological oedema. An effect on the normal cornea has been examined in a double blind study (to be published) revealing a quantitatively smaller but definite thickness reducing effect of tranexamic acid.

These investigations due to the antifibrinolytic effect of tranexamic acid lend support to the hypothesis that in man the fibrinolytic system is involved in the regulation of the normal as well as the pathological corneal thickness. During the last five years we have regularly followed graft thickness in the first 14 days after corneal transplantation. We report here an analysis of the course of graft thickness during this time period in various groups of corneal diseases and in a group of patients treated with tranexamic acid.

## Material and Methods

The material comprises 172 patients subjected to penetrating corneal transplantation during the period January 1st 1973 to April 1st 1978. Thirty of these patients were included in an earlier study of graft thickness (Ehlers 1974). The technique of operation and of measuring the graft thickness has been reported earlier (Ehlers 1974 Ehlers & Sperling 1977). Almost all the measurements were made by the authors. The graft thickness was measured several times during the 14 days of hospital stay after transplantation, and in a substantial number of cases it was measured almost every day.

The material has been divided into 6 groups: 1) 66 cases of keratitis (herpetic and non-herpetic); 2) 39 cases of keratoconus; 3) 23 cases of Fuchs endothelial dystrophy; 4) 17 cases of stromal dystrophy; 5) 16 cases of corrosion or mechanical lesion; and finally 6) 11 cases treated in the postoperative period by systemic tranexamic acid (daily amount 5 mg/kg body weight divided in three doses). This latter group comprises 9 cases of keratitis, 2 cases of Fuchs dystrophy, 4 cases of stromal dystrophy and 6 cases of mechanical lesion. Tranexamic acid treatment was started before the first postoperative day, often in cases with a fairly thick graft, as a therapeutic attempt. This explains the rather high values of graft thickness on the first postoperative day in this group. For each of the 6 groups average donor age and graft age were computed. Table I shows these data from which it may be seen that the groups are reasonably comparable. Analysis of variance revealed no significant difference between the groups in respect to donor age or graft age.

Table 1

An analysis of variance showed no heterogeneity among the age groups ( $F = 0.67$  for the donor age groups,  $F = 0.93$  for the graft age groups,  $F_1 = 5.4$ ).

Diagnostic group	No	Donor age (years) $\bar{x} \pm \text{SEM}$	Graft age (years) $\bar{x} \pm \text{SEM}$
Keratitis	66	44.93 $\pm$ 2.13	31.391 $\pm$ 4.27
Keratoconus	33	39.33 $\pm$ 3.12	31.13 $\pm$ 5.7
Fuchs dystrophy	23	40.52 $\pm$ 9.90	33.14 $\pm$ 4.46
Stromal dystrophy	12	40.83 $\pm$ 5.57	33.00 $\pm$ 1.1
Corrosion or mechanical lesion	16	45.94 $\pm$ 5.99	34.64 $\pm$ 2.7
Tranexamic acid treated	17	44.59 $\pm$ 4.27	33.94 $\pm$ 3.3

## Results

For each of the 172 patients the time course of the graft thickness was determined. Analysis of the curves in the 6 groups mentioned revealed three different responses. Fig. 1 illustrates the characteristics. Fig. 1 A is an example of graft thickness time course in a patient with keratitis. The steady fall is interrupted by a secondary rise beginning on the 5th postoperative day. After the 7th day a steady fall is again observed. Fig. 1 B shows the time course after grafting a patient with keratoconus. There is a steady fall during the observation 14 days and no secondary thickness increase around the 5th–6th day. Fig. 1 C shows the time course after grafting a patient with Fuchs endothelial dystrophy. The most remarkable in this curve is that the maximum thickness is found 2–3 days after the operation and not as in the other groups on the first postoperative day. Fig. 1 D shows the time course after grafting a patient with keratitis treated in the postoperative period with tranexamic acid. This curve shows a course similar to that observed in patients with keratoconus.

The four examples are considered to be characteristic. In order to document this statement average curves have been computed for the 6 groups mentioned (Figs. 2–7). Within each group the average of all available measurements on the days were calculated ( $\bar{x} \pm \text{SEM}$ ). In Figs. 5 and 6 the number of measurements on the last days were limited and therefore the data were added for several days and shown as value of the 11th day.

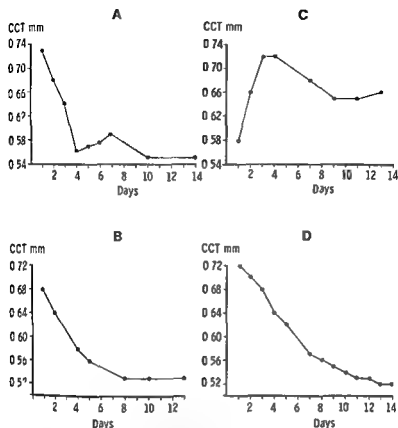


Fig 1

Graft thickness after penetrating keratoplasty Four examples of characteristic time course

- A Corneal host disease keratitis
- B Corneal host disease keratoconus
- C Corneal host disease Fuchs' endothelial dystrophy
- D Tranexamic acid treated keratitis

The 6 average curves show the three characteristic time courses. The groups of keratitis, stromal dystrophy and corrosion or mechanical injury (Figs 2, 5 and 6) all show a secondary thickness increase at the 6th day. The groups of keratoconus and tranexamic acid treatment (Figs 3 and 7) showed no secondary rise in thickness at the 6th day. The group of Fuchs' endothelial dystrophy (Fig 4) did not show the maximum thickness until the 3rd postoperative day.

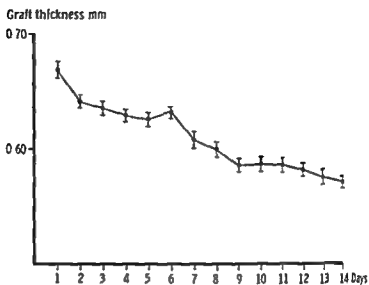


Fig 2

The average curve in the group of keratitis ( $\bar{x} \pm \text{SEM}$ )

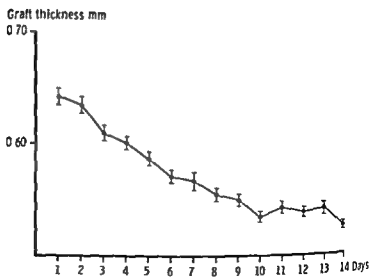
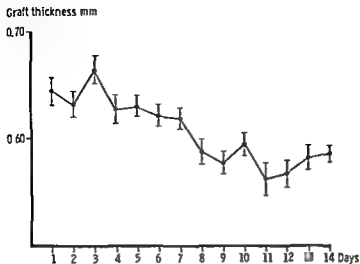
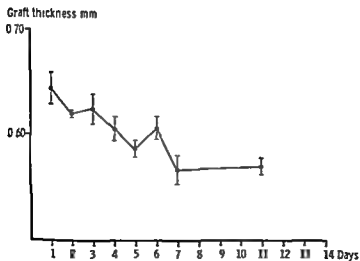


Fig 3

The average curve in the group of keratoconus ( $\bar{x} \pm \text{SEM}$ )



*Fig 4*  
The average curve in the group of Fuchs endothelial dystrophy ( $\bar{x} \pm \text{SEM}$ )



*Fig 5*  
The average curve in the group of stromal dystrophy ( $\bar{x} \pm \text{SEM}$ )



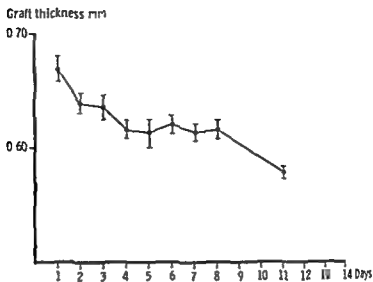


Fig 6

The average curve in the group of corrosion or mechanical lesion ( $x \pm \text{SEM}$ )

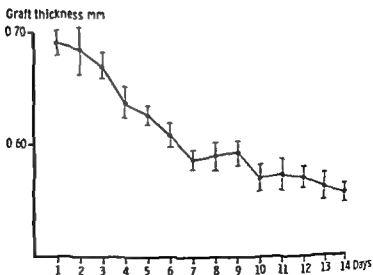


Fig 7

The average curve in the tranexamic acid treated group ( $x \pm \text{SEM}$ )

**Table II**  
Time course of postoperative graft thickness decrease

Diagnosis	No. of cases	Steady fall	Secondary increase 4th to 7th day
Keratitis	66	17	49
Keratoconus	38	30	8
Fuchs dystrophy	93	10	13
Stromal dystrophy	12	8	6
Mechanical lesion or corrosion	16	2	14
Tranexamic acid treatment	17	14	3
Total	172	79	93

43.5  $f=5$   $P < 0.001$

from the 1/2 curves it was counted how many showed a steady fall and how many showed a secondary rise within the 4th to 7th postoperative day. It is shown in the time courses of Fig. 1 A and B respectively. The results appear in Table II. Of the 66 cases of keratitis 49 showed a secondary rise and in the group of 38 cases of keratoconus only 8 showed a secondary rise. In the tranexamic acid treated group only 3 of 17 showed a secondary rise. Evaluation of the figures by  $\chi^2$  test showed that the observed frequencies differed significantly from a random universal distribution ( $P < 0.001$ ).

### Discussion

In a previously published study (Ehlers 1974) the graft thickness was followed in 10 patients. 32 of these with regular measurements. The purposes at that time was to show the changes in graft thickness in the total series of patients and not any differences between various groups. It was noted however that two types of postoperative graft thickness course occurred: one showing a steady fall and another with a secondary rise after about one week. There were an approximately equal number of cases in the two groups.

The present study has confirmed the existence of these two different postoperative thickness time courses. Patients with keratitis with stromal dystrophy or with corrosion or mechanical lesion show a secondary transient rise 4 to 7 days after the transplantation while patients with keratoconus show no such secondary rise.

As a new observation it has been noted that patients with Fuchs endothelial dystrophy show a different course characterized by an increase in graft thickness during the first postoperative days. When a secondary rise occurs in this group it tends to be some days later than in keratitis. Patients treated with the antifibrinolytic drug tranexamic acid show a course almost identical to that of keratoconus patients. It may be emphasized that the tranexamic acid treated group contains no cases of keratoconus.

The six groups did not differ with regard to donor age or graft area, and it is therefore tempting to seek an explanation of the different time courses in different responses to surgical trauma in the groups.

Ygge (1970) followed the plasma content of fibrinolytic inhibitors after abdominal surgery. He found that the antifibrinolytic activity was increased shortly after the surgical trauma and reached a maximum value after 3-4 days. In the next days the inhibitory effect fell and was minimal after about 1 week. The same time course was observed in patients subjected to injuries (Rasmussen & Saldeen 1970). Ygge (1970) also investigated the spontaneous fibrinolytic activity in serum and found a fall the day after the surgical trauma followed by an increase after 3-4 days occurring simultaneously with the fall in inhibitors.

These changes in fibrinolytic factors are understandable when the mechanism of wound healing is considered. A wound is primarily closed by fibrin, which at first forms the only stabilization of the wound. When vessels grow into the fibrin layer in 4-5 days the fibrin must be removed to allow the formation of collagen. It can therefore be considered appropriate to have a low fibrinolytic activity in the first days after a lesion and a high activity after 5 days when fibrin should be removed.

Reme & Witmer (1974) showed that fibrin is formed in iris wound and begins to disappear on the 5th day and is completely removed 7 days after the lesion.

The fibrinolytic system is therefore also active in the eye and the response with stabilization and later break down of fibrin would seem to be the same elsewhere in the body. Studies on traumatic hyphaema (Bramsen 1976) have shown that the secondary haemorrhages are prevented by administration of the antifibrinolytic drug tranexamic acid.

It would seem therefore that an increase in fibrinolysis locally in the eye as well as generally in the body occurs about 3-6 days after a lesion. This

ased fibrinolytic activity occurs simultaneously with or shortly before an  
rease in corneal thickness

A common response in postoperative graft thickness is observed in patients  
h keratitis stromal dystrophy and corrosions and mechanical lesion The  
neal thickness is maximal the day after the operation and falls steadily until  
5th day when a secondary and temporary increase occurs This increase is  
er a few days followed by a continuation of the steady fall This response  
rees with the variations observed in fibrinolytic activity in plasma after other  
uma The prevention of the secondary increase in thickness by antifibrino  
ic therapy supports this explanation The postoperative graft thickness course  
th a transient secondary increase 3 days after operation may be considered  
normal response This response can be altered by antifibrinolytic treatment  
The graft thickness course in keratoconus patients is identical with that ob  
ved after tranexamic acid treatment The secondary rise in thickness is  
sent or possibly delayed It is well known that corneal grafting in kerato  
us gives very favourable results We have noted that the graft in kerato  
nus eyes often becomes very thin The thickness time course in this group was  
derstandable if the patients with keratoconus had a low level of fibrinolytic  
tivity or an abnormal weak fibrinolytic response to injuries

The graft thickness time course in patients with Fuchs endothelial dystrophy  
fers from all the other groups in the sense that the maximal graft thickness  
not gained until three days after the operation and not until after seven  
ys does the thickness show a fall In a previous study on Fuchs dystrophy  
ramsen & Ehlers 1971) it was shown that the corneal oedema could be re  
iced by means of antifibrinolytic treatment The graft thickness time course  
uld be understood if these patients opposite to keratoconus patients had a  
gh level of fibrinolytic activity or an abnormally large or prolonged fibrino  
tic response to injuries We are at present studying the variations in anti  
brinolytic factors in serum after corneal grafting (Bramsen & Stenbjerg 1979)  
hese patients have been selected because they routinely stay in hospital for  
o weeks and thus give the opportunity to follow changes by daily blood  
mples There is nothing however to suggest that the secondary rise in thick  
ss is confined to transplantations Mishima (1965) included a curve of corneal  
ickness after cataract extraction clearly showing a transient increase about  
ne week after operation

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## HUMAN CORNEAL ENDOTHELIUM IN ORGAN CULTURE

### The Influence of Temperature and Medium of Incubation

BY

STEFFEN SPERLING

Forty four human corneas from patients between 21 and 86 years were incubated in Eagle's minimum essential medium with Earle's salts 10-46 h post mortem. The influence of incubation temperature and composition of the medium on endothelial survival was evaluated. Whole corneas were stained by alizarine red. Recent cell loss was indicated by morphological alterations in the endothelial pattern. After 20-28 h of incubation minimum cell loss was found at 31°C when 8% Dextrane 250 and 20% serum or 8% Dextrane 500 and 10% serum was added to the medium.

*Key words:* alizarine red - dextrane - endothelium - human cornea - morphology - temperature - organ culture

This study was undertaken when it was realized that the endothelium on whole human corneas incubated in organ culture under conditions as described by Summerlin et al (1973) showed signs of continuous cell death (Sperling 1978). The aim of the present study was to evaluate the influence of varied incubation procedures. Evaluation was based on recognition of morphological alterations in the endothelial cell pattern after cell loss.

Normal endothelial cells form joint meetings of three. Each cell is surrounded by 4-8 neighbouring cells. The borders between neighbouring cells meet at angles approximating 120°. Pointed angles are extremely rare. During incubation in tissue culture medium damaged cells are expelled from the coherent cell sheet by expanding neighbours. Four or more expanding cells form joint meetings. In minutes to hours these cells slide and form joint

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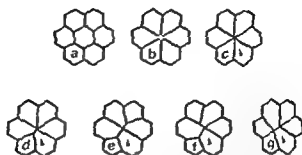


Fig 1

a-c Formation of joint meeting of six originally hexagonal cells d-g Transform to joint meetings of four cells Arrows indicate reformation figure centres

meetings of three or four. During reformation of joint meetings of three cells appear in characteristic patterns shown in principle in Figs 1 d g (Starrard 1976 Olson et al 1978 Sperling 1978)

In each pattern Figs 1 d g two or more cells with converging pointed or one cell with a pointed angle plus a joint meeting of four occur. A pair of cells appearing in one of these configurations (in the following reformation figures) was regarded as indication of recent loss of one cell.

## Material and Methods

Whole human eyes were enucleated from cadavers stored at  $13-20^{\circ}\text{C}$  for 8-10 h later at  $10^{\circ}\text{C}$ . Hypotonic eyes with indented corneas or with contact between rim cornea were rejected. After rinsing in tap water corneas were excised with a 12 mm rim of sclera, and the endothelium was stained for one min by sterile 0.5% trypan blue in 0.9% NaCl. Forty four corneas in which less than one per cent donor endothelial cells were found in unfolded areas (Sperling 1978) were suspended in 100 ml glass infusion bottles by a suture through the scleral rim and incubated in 25 ml of tissue culture medium (Fig 2). Patient age and time between death and incubation is indicated for each cornea in Fig 3.

The basic medium was composed of Eagle's minimum essential medium with Earle's salts, L-glutamine (0.092 mg/ml),  $\text{NaHCO}_3$  (2.2 mg/ml), ampicillin (Bristol) 0.1 mg/ml, carbenicillin (Astra) 0.5 mg/ml and nystatin (Squibb) 50 IU/ml. Ten or 50 per cent foetal calf serum (Microbiological Ass.) and Dextran (Pharmacia) with molecular weights 50 000, 250 000 or 500 000 were added to the medium. The bottles were inflated with a gas of 35 per cent air and five per cent  $\text{CO}_2$  and placed at  $37^{\circ}\text{C}$  temperature.



Fig 2

excised cornea suspended by a suture through the scleral rim in 9.5 ml of medium in a 100 ml glass infusion bottle

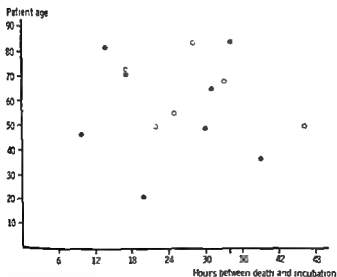


Fig 3

patient age and time between death and incubation indicated for each of the forty four cadaver corneas included in this study. An open circle represents one cornea. A dot represents two corneas from the same patient.



After 20-28 h of incubation the corneas were rinsed briefly in minimum essential medium and the endothelial cell borders were stained for four min by 0.1% trypan blue 10% saccharose in deionized water (Sperling 1971). Stained whole corneas placed epithelium down on microscope slides and filled by minimum essential medium. The flattened central 6 mm of the endothelium was studied in a light microscope fitted with a photo tube. Objective Zeiss 10x Film Polaroid 66, 55x105 mm.

The microscope was focused at random in the central flattened area and 10 photographs were obtained of each cornea. In the experiments on temperature dependence a photograph was obtained when 3/4 or more of a full microscope field could be brought in focus. Some folding of the membrane of Descemet was present at the beginning of the experiments. Increased corneal thickness during incubation in media without Dextrane led to progressive endothelial folding. Many reformation figures appeared on the slopes of endothelial folds and in rows parallel to folds. These reformation figures were excluded from the estimates of cell damage by the procedure of test area selection. In experiments with Dextrane in the medium less folding occurred and the microscope was focused at random in the central area. On each photograph one small and three large frames were drawn. The total number of cells was counted in one frame equivalent 0.030 mm<sup>2</sup> on cornea and the number of reformation figure centres were counted in three frames each equivalent 0.06 mm<sup>2</sup> on cornea. Centres of reformation figures were determined as indicated by arrows in Fig 1. The frames were drawn and the numbers of cells and reformation figure centres per frame were counted according to the rules described by Sperling & Gundersen (1978). By this procedure the total number of cells were counted in 12 areas per cornea and the number of reformation figure centres were counted in 36 areas. Counts from the same cornea were averaged and the number of reformation figures were expressed in per cent of the number of cells per area.

## Results

Twenty corneas were incubated in the basal medium with 10% calf serum at temperatures between 19 C and 37 C (indicated temperature  $\pm 1$  C). Fig 4 the percentages of reformation figures after 20-28 h of incubation related to temperature. Minimum cell damage occurred between 23 C and 34 C. The cellular morphology after 20-28 h of incubation at 31 C is illustrated in Figs 6 & 7.

In the preceding experiments increase in the corneal thickness during incubation lead to progressive endothelial folding. In four corneas incubated in 5% Dextrane 50 to the medium reduced but did not abolish the progressive folding. Four corneas were incubated at 31 C with eight per cent Dextrane 250 added to the medium. Slight endothelial folding was still present after incubation but reformation figures did not accumulate in relation to folds on these corneas. Folding was minimal in four corneas incubated in eight per cent Dextrane 500.

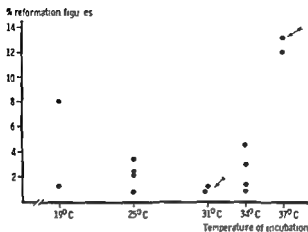


Fig 4

formation figures after 20-28 h of incubation in per cent of the total number of cells per area related to incubation temperature. Arrows indicate corneas shown in Figs 6 & 7.

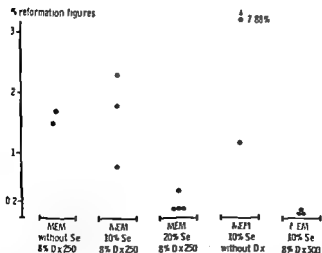


Fig 5

formation figures in per cent of the total number of cells per area after 20-28 h of incubation in Eagle's minimum essential medium with Earle's salts (MEM) foetal calf serum (Se) and Dextrane (Dx) at 31°C.

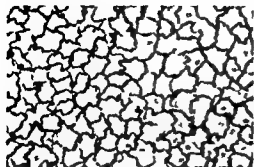


Fig 6

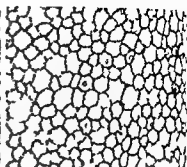


Fig 7

Fig 6 Endothelial cell borders stained by alizarine red after 22 h of incubation 37°C From the cornea marked with a single arrow in Fig 4 Magnification 150x

Fig 7 Endothelial cell borders stained with alizarine red after 26 h of incubation 31°C From the cornea marked with a double arrow in Fig 4 Magnification 150x

The percentages of reformation figures after 20-23 h of incubation at 31°C in the basal medium without serum with 10 per cent serum with 10 per cent serum without Dextrane with Dextrane 250 and with Dextrane 500 are indicated in Fig 5 Four corneas were included in each of these experiments. Less than one per cent of reformation figures was found when 10% serum and 8% Dextrane 250 or 10% serum and 8% Dextrane 500 was added to the medium.

## Comments

In this study corneas were prepared for incubation 10-46 h post mortem. The endothelium was extremely sensitive to mechanical trauma during enucleation and preparation of the corneas. Most eyes with post mortem times of more than 36 h were rejected because of corneal indentation. Only corneas with less than one per cent blue stained endothelial cells were incubated. It appears from Fig 3 that patient age and post mortem time were not correlated. This indicates that the state of the endothelium can not be predicted from post mortem time from patient age or from a combination of the two.

An earlier study (Sperling 1978) indicates that a complete reformation of the endothelial cell pattern occurs within hours after cell loss. If this observation is correct the percentage of reformation figures will be related to actual endothelial death rate and independent of initial cell density and initial trauma damage left undetected by trypan blue staining.

The present study indicates that an incubation temperature at or below temperature *in vivo* is essential for endothelial survival *in vitro* (Fig 4). Parallel observations were made on rat oral mucosa and epidermal cells *in vitro* (Jepsen 1974; Indo & Wilson 1977).

The basic medium comparable to the medium used in the present study (Dextrane 40) was found to be toxic to rabbit endothelium at 37°C (Geeracis et al 1977) and to human endothelium at 25°C (Sachs et al 1978). In the present study the least amount of cell damage was found when eight per cent of Dextrane 250 or Dextrane 500 was added to the medium of incubation at 37°C. Eight per cent was an arbitrary choice. Experiments on prolonged incubation in media containing Dextrane are in progress. They may show whether the observed positive effect of Dextrane is merely an initial phenomenon caused by a lower initial swelling rate.

### Acknowledgments

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# CORNEAL ENDOTHELIAL CELL DENSITY IN IRIDOCYCLITIS

BY

KIRSI SETÄLÄ

The endothelial cells of both eyes of 60 unilateral iridocyclitic patients were photographed with a specular microscope to establish the possible effect of iridocyclitis on corneal endothelial cells

Chronic severe iridocyclitis with mutton fat keratic precipitates (KP) lowered the central endothelial cell count Five patients out of the seven with mutton fat KPs had a distinctly lower central cell density in the affected eye In the remaining patients no significant difference in cell densities could be demonstrated between affected and healthy eyes Neither the inflammatory process itself nor the round white KPs had a deleterious effect on the central corneal endothelial cell densities

*Key words:* iridocyclitis - keratic precipitates - corneal endothelial cell density - endothelial cell loss - clinical specular microscope

The corneal endothelium is a delicate tissue which is susceptible to changes in environment Specular microscopy provides a mean for direct photographic examination of the *in vivo* corneal endothelium at relatively high magnifications

In an earlier paper (Setälä & Vannas 1978) we reported the difference in corneal endothelial cell counts between the affected and healthy eyes of Posner Schlossman patients The main factor affecting the endothelial cell density seemed to be the intermittently high IOP (Vannas et al 1977) The other possible detrimental factors in the Posner Schlossman patients were keratic precipitates (KP) and aqueous changes This led us to study iridocyclitic eyes to elucidate the effects of KPs and aqueous changes on endothelial cell density

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Earlier studies of enucleated eyes revealed some differences of opinion concerning the effect of iridocyclitis on corneal endothelial cells. Stocker assumed that the inflammatory process as such had a deleterious effect on endothelium and Irvine (1956) observed localized flattening and atrophy of the endothelium under KPs.

In the present study we examined and photographed both eyes of 60 lateral iridocyclitic patients with a specular microscope and compared endothelial cell counts between eyes of the same patients.

## Material and Methods

The series comprised 60 patients in the Department of Ophthalmology, University of Helsinki (Fig. 1). There were 32 women and 28 men. The ages of the patients ranged from 11 to 74 years with a mean of 41.4 years. Every patient had unilateral iridocyclitis with no history of inflammation in the other eye which was externally and internally normal. The contralateral healthy eye served as control. Both eyes had normal intraocular pressure. The underlying aetiological disease was ankylosing spondylitis in six cases, rheumatoid arthritis in six cases and sarcoidosis in two cases. One patient had Reiter's syndrome. In the remaining cases the aetiology was unknown (Table I).

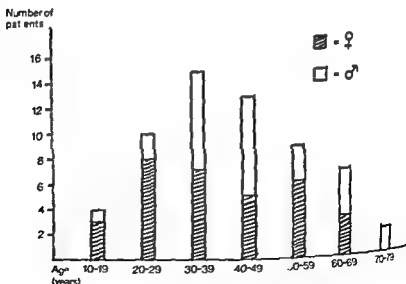


Fig. 1  
Age and sex distribution of patients

Patients were divided into three groups according to their signs and symptoms (Table II A B C) (Hogan et al 1959). Seven patients had mutton fat keratic precipitates, 35 had round white KPs, and the third group (18 patients) displayed no definite KPs during the examinations.

The endothelial cells were photographed with a specular microscope. Several series, five on average, were taken of the central cornea of both eyes. The morphology, size, and cell density were analyzed and counted as described in the previous paper (Vannas et al 1977).

*Table I*

Aetiology of iridocyclitis	
Ankylosing spondylitis	6
Rheumatoid arthritis	6
Sarcoidosis	9
Reiter's syndrome	1
Others	45
	60

*Table 2 A*  
Patients with mutton fat KPs

Sex	Present age / age at onset (years)	Recurrences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm <sup>2</sup> )		
					affected eye	control eye	difference (%)
m	49/49	3 x	++	+	2401	2931	+19.5
m	15/19	chronic	+	-	3064	3519	+15.4
m	72/90	chronic	+	-	9650	3105	+14.7
f	34/24	> 10 x	++	-	9650	3064	+13.9
f	49/26	chronic	++	+	2434	2815	+11.8
f	11/9	chronic	+	-	3183	3271	+ 2.5
m	50/49	5 x	++	+	1937	1937	0
							+11.4% mean



**Table 2 B**  
 Patients with small or medium sized round white KPs

Sex	Present age/age at onset (years)	Recurrences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm)		
					affected eye	control eye	diff. (%)
f	27/26	3 x	++	-	2950	3146	+3
f	39/40	2 x	+	-	2950	3100	+3
f	28/23	> 10 x	++	-	3090	3230	+4
m	63/52	5 x	++	+	2836	2939	+3
m	31/29	> 10 x	++	+	2740	2970	+3
m	40/33	3 x	+	-	2567	2650	+3
f	26/26	no recurrences	+	-	2930	3040	+3
f	31/29	3 x	+	-	2483	2536	+1
m	48/40	> 10 x	++	+	2432	2770	+14
f	29/21	3 x	+	-	3100	3146	+1
m	49/40	6-7 x	++	+	2492	2443	-2
m	32/2	6 x	+	-	2732	2710	-1
f	22/22	no recurrences	+	-	2770	2790	+0
f	60/60	chronic	+	-	2950	2980	0
m	51/43	> 10 x	++	+	2150	2150	0
m	61/58	6-7 x	++	+	2698	2698	0
m	24/18	5 x	++	-	2939	2939	0
f	50/52	5 x	+	-	2627	2677	0
m	74/ 2	2 x	++	+	2815	2815	0
f	24/22	2 x	++	+	3105	3100	0
m	44/42	3 x	+	-	2815	2815	0

## Results

The endothelial cell densities of the healthy and affected eyes are presented in Table II A B and C.

Seven patients had a chronic severe iridocyclitis with only intermittent quiescent periods. These eyes had mutton fat KPs. Five of these patients displayed a distinctly lower central endothelial cell density in the affected eye.

Table 2 B (cont)

Present age/age at onset (years)	Reurrences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm <sup>2</sup> )		
				affected eye	control eye	difference (%)
40/40	no reurrences	++	-	2931	2931	0
57/57	no reurrences	++	-	3105	3105	0
40/39	no reurrences	++	+	2650	2650	0
32/32	no reurrences	++	+	2931	2931	0
62/48	6 x	++	-	2194	2194	0
73/43	2 x	++	-	2120	2120	0
36/34	3 x	+	-	3015	3015	0
45/2	> 10 x	+	-	3312	3312	0
32/30	8 x	++	-	2788	2770	-0.6
52/49	2 x	++	-	2608	2567	-1.0
31/31	no reurrences	+	-	3353	3312	-1.2
31/2	6-7 x	++	-	2900	2860	-1.4
3/30	5-6 x	++	-	3072	2930	-1.4
66/2	> 10 x	++	+	2730	2690	-1.5
patients						+0.7% mean

the healthy eye. The mean IOP of the affected eyes was 12 mmHg and of control eyes 14 mmHg.

The second group of patients had round white KPs. The cell density was lower in the iridocyclitic eye of 13 patients but the same in both eyes of 16 patients. In six eyes the endothelial cell density was slightly lower in the healthy than in the iridocyclitic eyes. The mean difference was 0.7%. The mean IOP was 13 mmHg in the affected eyes and 16 mmHg in the control eyes.

Table 2 C  
Mild iritis with no discovered KPs

Sex	Present age/age at onset (years)	Recur rences	Aqueous flare	Fibrin	Endothelial cell density (cells/mm)		
					affected eye	fellow eye	differe nce (%)
f	44/44	no recur rences	+	-	2650	2730	+3.0
m	34/34	no recur rences	+	-	2677	2737	+2.0
f	51/50	3 x	+	-	2319	2360	+1.7
m	49/49	no recur rences	+	-	2401	2443	+1.7
f	43/42	> 10 x	+	-	2490	2490	0
f	22/18	2 x	+	-	2900	2900	0
f	18/7	2 x	+	-	2737	2737	0
f	25/19	2 x	++	-	3146	3146	0
m	34/31	no recur rences	++	+	2915	2915	0
f	16/16	no recur rences	++	+	3395	3395	0
m	44/44	no recur rences	+	-	2737	2737	0
f	30/30	no recur rences	++	+	2990	2990	0
m	36/36	no recur rences	++	+	3694	3694	0
f	62/62	no recur rences	+	-	2150	2150	0
f	64/64	no recur rences	++	+	2791	2763	+1.2
f	34/33	3 x	++	-	3110	3120	+0.3
f	59/51	3 x	+	-	2070	2079	+0.4
f	59/57	4 x	++	-	3064	2931	+4.5
18 patients							+0.64 mean

In the third group of 18 patients no KPs were seen at the time of photography and only a few small KPs or debris had previously been noticed on the endothelial surface. Only four patients had a lower cell density in the affected eye. Ten patients had the same cell density in both eyes and four had a lower cell density in the healthy control eye. The mean IOP was 12 mmHg in the affected eyes and 15 mmHg in the control eyes.

These three groups are rather small precluding statistical comparison. An interesting result is that in the first group of patients with mutton fat KPs and severe iridocyclitis 71% of the affected eyes had a lowered cell density compared with the healthy eyes. In the second group with white KPs there was some tendency towards a lower cell density in the affected eyes (+0.7%) and in the third group in which no KPs were detected no difference could be shown between affected and healthy eyes.

#### *Correlation with duration of disease*

Seventeen patients had had only one attack of 1-3 weeks in duration. These patients were photographed at the end of the attack. No clear difference was noticed between eyes of these patients.

Twenty nine patients had had two or more iridocyclitic attacks before photography. Only four of these patients had a history of less than two years' iriditis and in the remaining 25 patients two or more years had elapsed between the first attack and the time of photography. A small tendency towards lowered endothelial cell density was discovered in these patients. None of the patients showed a clear difference between healthy and affected eyes.

Fourteen patients had a chronic disease or several recurrences with only short healthy intervals. Twenty nine per cent of these patients had distinctly lower cell counts in the affected eyes. The lowered endothelial cell densities correlated well with mutton fat KPs: every patient with a clearly lower cell density also had mutton fat KPs.

#### *Morphology of the endothelial cells*

The diseased eyes with a lower cell density had large cells and showed a more irregular cellular pattern than the control eyes which showed a regular mosaic pattern (Fig. 2).

### **Discussion**

Patients with mutton fat KPs displayed a lowered endothelial cell count in the affected eye in 71% of the cases. Round white KPs or the inflammatory process per se did not seem to influence the central corneal endothelial cell

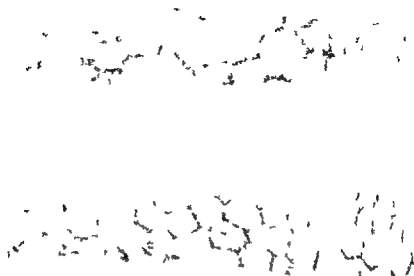


Fig 2

Large somewhat hazy irregular endothelial cells in iridocyclitic eye with many KPs (A) compared with the normal endothelium of the healthy fellow eye of the patient (B)  $\times 350$

density. These results were obtained by photographing the central corneal endothelial cells of 60 unilateral iridocyclitic patients with a specular microscope and comparing the healthy and affected eye of the same patients. Previous investigators have found the cellular pattern to be similar in both eyes of normal individuals. Kaufman et al (1966) examined 180 normal patients and noted that 98% had an identical endothelial cell pattern in both eyes. Various patients in this study received differing therapeutic regimens but no particular mode of therapy could be specially correlated with endothelial cell loss.

A major limitation of the method is that only a small area of the endothelium is photographed with each exposure. Further, the same area cannot be found later, and our method does not allow us to select areas under KPs for photography. Areas under the KPs could be seen only hazily and could not be well documented. It is our impression that KPs, by covering the endothelial cells, inhibit the cell reflexes that are photographed by the endothelial camera. That is why the exact cell number and cellular survival under the KPs cannot be determined.

If cell loss takes place in the lower part of the cornea it should be reflected in the cell count of the central area because healing occurs by sliding rather than by cell multiplication (Bourne & Kaufman 1966 Kaufman & Katz 1966). In another study Blackwell et al (1966) compared central endothelial cell numbers with peripheral cell counts. No differences were found between central superior temporal and inferior areas of the cornea (except in their intraocular lens group). One less studied variable is the change in relative cell densities with time. No information is available concerning the length of time required after a profound loss of endothelial cells in one area before other cells spread and equalize cell counts in the affected and peripheral areas. In our study 16 patients were photographed after their first iridocyclitic attack but in the remaining cases enough time had elapsed for endothelial cell spread to occur before photography.

The patients with mutton fat KPs displayed a lowered endothelial cell count in the affected eye. This is in agreement with previous studies in which epithelial edema was often noticed in areas corresponding to KPs (O'Connor 1962). Attenuation and localized flattening of the endothelium under KPs has also been observed in long standing iridocyclitis (Irvine 1956). Inomata & Smelser (1960) produced uveitis by intravitreal injection of bovine serum albumin into albino rabbits. The inflammation caused corneal thickening and haziness. Ultrastructural endothelial changes included mononuclear cell infiltration and endothelial vacuolization. Many of these invading cells were located between the endothelium and Descemet's membrane but the endothelial cell cytoplasm showed little evidence of change. It is especially noteworthy that the apical junctional complexes of the endothelial cells could easily open to admit invading cells and reform after these cells had entered. The greatest changes in the endothelium occurred in the area of pre-precipitates.

Based on the above it appears that temporary endothelial damage and functional changes may occur in experimental iridocyclitis of relatively short duration in rabbits but actual cell loss is not a general finding. These studies agree with our clinical findings. Patients with recurrent attacks of nongranulomatous uveitis did not show interocular differences in endothelial cell densities between healthy and affected eyes. In contrast patients with mutton fat keratic precipitates and chronic disease showed distinctly lower central endothelial cell densities in their affected eyes.

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## PRIMARY LOCALIZED ORBITAL AMYLOIDOSIS

BY

JENS HENRIK NEHÉN

The present paper reports a case of primary localized amyloid tumour of the orbit in a 65 year old woman in whom myasthenia gravis had been diagnosed 33 years before. An amyloid tumour with this location is very rare, and up to the present time only 13 cases have been recorded in the literature. The reported orbital amyloid tumour revealed a considerable accumulation of activity at orbital scintigraphy with  $^{99}\text{Tc}^m$  and compared with the conventional pseudotumours a distinct rise in density at computed tomography after intravenous infusion of iodized contrast medium.

*Key words:* amyloidosis - orbital amyloidosis - orbital tumour - myasthenia gravis -  $^{99}\text{Tc}^m$  scintigraphy - computed tomography

amyloid is a fibrous protein which is collected in the ground substance between the cells and fibres. It most notably involves the walls of capillaries and arterioles and the surrounding tissues. The most useful histological test for amyloid is staining with Congo red. It stains collagen and elastic tissue as well as amyloid, but all forms of amyloid, regardless of its location, reveal a green dichroism and birefringence when viewed in a polarizing microscope.

The main amyloid protein is a microfibril which possesses properties distinct from those of any other known mammalian protein. These include a characteristic appearance on electron microscopy and a characteristic X-ray diffraction pattern with the fibrils consisting of polypeptide chains arranged in an antiparallel conformation with a  $\beta$  pleated structure (Glennner et al 1973).

There is no general agreement as to the best classification of the various

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Table 1

	Sex	Age (years)	Duration of history	Proptosis	Limitation of ocular movements	Localization
Pollems (1920)	♀	42	10	+right -left	+right -left	behind both the superior and the inferior orbital margin of the right orbit and behind the superior orbital margin of the left orbit
Handoussa (1954)	♂	45	1	+	+	upper and inner quadrants of the left orbit
Easton et al (1961)	♀	49	4	+	+	inner part of the right orbit outside the muscle cone
Groniowski et al (1965)	♀	51	5	+	+	lower and outer quadrants of the left orbit
Howard (1966) case 1	♀	21	2	-	+	the region of the left lacrimal gland
case 2	♂	60	4	+	+	upper and inner quadrants of the right orbit
Kassman et al (1967)	♀	53	12	+	+	behind the inferior orbital margin of the right orbit
Raab (1970)	♂	55	9	+	+	behind the left superior orbital margin and in upper and inner quadrants of the right orbit
Radnot et al (1971)	♀	62	<1	-	-	the region of the right lacrimal gland
Schubert (1972)	♀	18	2	+	-	lower and outer quadrants of the right orbit
Knowles et al (1975)	♀	61	2	-	-	the regions of both lacrimal glands
Jensen (1976)	♀	49	1	+	+	inner parts of both orbits
Savino et al (1976)	♂	51	1	+	+	upper and outer quadrants of the right orbit
Nehen (1978)	♀	65	2	+	+	behind the superior orbital margin of the left orbit

Table 1

Consistence	Attached to bone	Lymphocytes	plasma cells	Foreign body giant cells	Electron microscopy	Amyloid infiltration of vessel walls	Partial excision	Total excision	Recurrence	Capsule	Positive anti nuclear factor
hard	+	+	+	+		=	+right		+		
soft	+	+		+		+	+			-	
hard	+	+	+	+		+	+		+	-	
hard		+	+	+		+	+		+	-	
	+			-		+		+			
hard				+		+	+		+	-	
hard	+					+	+		-		
hard			+			+	+left			-	
					+	+		+			
hard						+		+			
hard		+	-		+	+	+				
hard		+	+	+	+	+		+right			
hard		+	+	+			+				+
hard	+	+	+	+		+	+		+	-	+

manifestations of amyloidosis. The one suggested by Reimann et al. classified amyloidosis as primary when there was no underlying disease, secondary when there was an associated chronic disease as tumour, but when small localized masses of amyloid were found and as amyloidosis associated with multiple myeloma in the presence of the latter disease. This generally remained as the major clinically operational classification.

Primary localized orbital amyloidosis appears to be extremely rare and isolated case reports account for the principal literature. The pathogenesis is unknown and the relationships to the other patterns of amyloidosis are uncertain.

Up to the present time only 13 cases of primary localized orbital amyloidosis have been recorded in the literature. These cases are surveyed in Table I. In an additional case is presented.

### Case Report

A 68 year old woman in whom myasthenia gravis was diagnosed in 1944 has been under treatment with neostigmine. In 1946 she received X-ray irradiation to the thymus (3000 r). In 1948 right sided maxillary and frontal sinusitis was treated by osteotomy of the right maxilla. In 1971 resection of the right maxillary sinus was performed; it was filled with fibrous tissue which did not contain amyloid. In 1972 a marked swelling around the left eye suddenly developed; it was accompanied by moderate impairment of vision which has persisted unchanged afterwards. After some time the patient had recurrent episodes of pain and lacrimation in the left eye.

In 1974 at the age of 65 years the patient was treated for the first time at the Department of Ophthalmology, University Hospital, Århus. Pronounced proptosis (Hertel exophthalmometer: right eye 12 mm, left eye 21 mm), downward displacement, slight ptosis, loss of upward movement of the left eye and diplopia were noted. At the temporal two thirds of the supraorbital margin a hard, immobile tumour was palpated; it extended posteriorly between the orbital roof and the eyeball and adhered to the underlying bone. Ophthalmoscopy revealed a slight impression and elevation of the upper half of the retina caused by compression by the tumour. Visual acuity: right eye 1.0, left eye 0.4. Incipient cataract of the left eye. Applanation tonometry: right eye 12 mmHg, left eye 17 mmHg. No visual field defects.

Tomography of the left orbit showed blurring of the soft structures but no destruction or hyperostosis. Left sided orbitophlebography disclosed block of the anterior orbital vein anteriorly in the medial part of the orbit. Orbital scintigraphy showed a large accumulation of activity upwards in the left orbit (Fig. 1).

Partial excision was performed through an anterior orbitotomy with the removal of a piece of tissue measuring 1 × 2.5 × 3 cm from the anterior part of a large, greyish-red, slightly bleeding tumour situated between the orbital roof and the eyeball.

Histologically the tumour was made up of nodules of an amorphous eosinophilic material which showed a strongly positive reaction to amyloid staining with Congo red and distinctly dichroism in polarized light. The eosinophilic material was also filled up by collagen fibres. Scattered in the tissue were fairly large lymphocyte infiltrates.

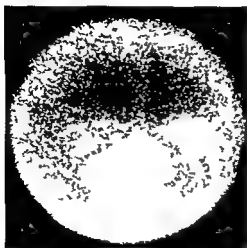


Fig 1

Orbital  $^{99}\text{Tc}$  scintigram. Large accumulation of activity upwards in the left orbit

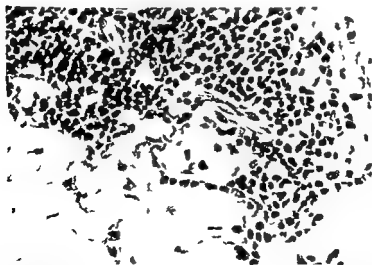


Fig 2

Amyloid deposits with scattered foreign body giant cells and in the connective tissue many lymphocytes and a few plasma cells. Haematoxylin and eosin  $\times 300$

and a smaller number of plasma cells. At the margins of the amyloid areas, few foreign body giant cells were present (Fig 2).

Biopsy specimens from the liver and rectal mucosa showed no signs of amyloidosis.

Immuno electrophoresis showed reduced amounts of IgA. Antinuclear factor was positive with medium reaction.

The erythrocyte sedimentation rate had been elevated since 1971 and was 41 mm at the time of operation.

Laboratory tests and clinical examination gave no basis for either systemic primary or secondary amyloidosis.

During the next few years periods with increasing protrusion and limitation of movements occurred. In 1977 tomography of the left orbit showed thinning of orbital roof and as before density of the soft tissue of the upper half of the orbit. Biopsy revealed an amyloid tumour of the same character as before. In 1980 protrusion of the left eye had increased to the same extent as before the first examination. Visual acuity was unchanged. No Bence Jones protein was present in the urine. Computed tomography disclosed a tumour superiorly and laterally in the left orbital cone. It was 1 cm in width and extended 2 cm posteriorly. There were exostosis and a speckled osseous structure of the lateral orbital wall at the level of the tumour. Density values in the tumour tissue were between 45 and 51 Hounsfield units before the injection of the iodinated contrast medium and between 60 and 80 units after injection (Fig 3). No treatment had been given.



Fig 3

Computed tomography. A tumour superiorly and laterally in the left orbital cone with exostosis of the lateral orbital wall and protrusion of the left eye (after contrast infusion).

## Discussion

Table I gives a survey over the reported cases of primary localized orbital amyloidosis.

The ages of the 13 previously described patients (9 women and 4 men) ranged from 21 to 78 years. Only four cases were bilateral. Apart from the lacrimal gland, the clinical picture was characterized by slowly progressing proptosis and limitation of eye movements, often associated with diplopia. With a single excision the tumours were hard and — in all cases in which information was available — firmly attached to the underlying bone and without a capsule. Common to all the cases is a histological picture with partial replacement of connective tissue by irregularly shaped aggregations of amyloid material. In most of the cases, marginal infiltration with foreign body giant cells, and arterioles invariably showed amyloid infiltration of their walls. In almost all of the cases there was infiltration of plasma cells and lymphocytes in the connective tissue. In only a few of the cases had total excision been possible without recurrence. These include all the amyloid tumours located in the lacrimal gland. In the cases where the amyloid tumours were large with extension deeper into the orbit and with more infiltrative growth, there was a marked tendency to recurrence and repeated local excision had often been required.

The cause of amyloidosis is not known. Several pathogenic mechanisms have been considered through the years.

The role of immune mechanisms in the genesis of amyloid is a topic that has been widely discussed and has resulted in a variety of opinions.

Benlender et al. (1963) are of the opinion that the amyloid fibrils are formed from immunoglobulin proteins. They have demonstrated that the major protein component of the amyloid fibrils both in cases of primary systemic amyloidosis and in isolated tumours composed of amyloid is an immunoglobulin light polypeptide chain and/or its amino terminal fragment.

The role of the reticulo endothelial system in the pathogenesis of amyloid has also received considerable attention. According to experimental studies of Jellum (1964) amyloidosis is the result of a persistent antigenic stimulation of mesenchymal tissue leading to a break down or a perversion of the protein synthesizing function of the reticulo endothelial system. His two phase cellular theory of the formation of amyloid consists of a pre amyloid phase marked by increased proliferation of pyroninophilic reticular and lymphoid cells. The second phase is the actual amyloid phase characterized by a suppression of proliferating pyroninophilic mesenchymal cells and the appearance of PAS positive cells. These cells secrete an abnormal protein deposited locally as amyloid.

Although the significance of the immune apparatus in the pathogenesis of experimental amyloidosis can be considered an established fact, the extent to which its functional state may influence the development of amyloidosis is not clear. The role of the thymus was studied by Ranlov (1966). He showed that in mice previously subjected to thymectomy or combined thymectomy and lethal irradiation experimental caseinate amyloidosis developed more rapidly and to a greater extent than in control animals. He concluded that thymectomy tends to promote the development of amyloid disease perhaps through the elimination of a superior function of the thymus in regulating the protein synthesis of mesenchymal tissue. In the absence of the thymus even short but antigenic stimulations can be imagined to lead to a break down of the mesenchymal tissue function with subsequent deposition of amyloid. In Ranlov's investigations immuno electrophoresis was not employed but Arnason (1964) reported a subnormal level of IgA in thymectomized mice.

The cases reported by Gronowski et al (1965) and Jensen (1966) were discussed in the light of Teitelbaum's two phase cellular theory of the formation of amyloid and in both the two phases could be observed. Furthermore, in Jensen's case the presence of many IgG carrying lymphoid cells could confirm Glenner's theory of the immunoglobulin origin of amyloid. Both were of opinion that their cases could be a reaction to some immunological process, perhaps an auto immune reaction.

In the present case it is noteworthy that the patient had for many years suffered from myasthenia gravis which is generally considered an auto-immune disease. In view of Ranlov's work on the role of thymectomy in the development of amyloidosis it is also of interest that the patient had been treated with irradiation of her thymus.

The reported orbital amyloid tumour revealed a considerable accumulation of activity at orbital scintigraphy which also can be demonstrated in orbital pseudotumours (Heuer & Lihers 1972) but compared with the conventional orbital pseudotumours there was a distinct rise in density at computed tomography after intravenous contrast infusion.

According to Wackenheim et al (1977) the density of a pseudotumour on computed tomography is practically unchanged after intravenous infusion of iodized contrast media. On the other hand Gyldensted et al (1977) reported a rise in density values corresponding to an average of 14.4 Hounsfield units. Before contrast infusion the density of orbital pseudotumours does not differ essentially from several other orbital tumours and a differential diagnosis is not possible.

The present paper is the first in which a description of both computed tomography and orbital scintigraphy of an orbital amyloid tumour is given, for which reason it is not possible for any comparison to be made.

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## POSTMORTAL 'VITAL' STAINING OF THE EXTERNAL EYE

BY

M S NORN

Two hundred and two eyes from 112 individuals were vital stained from 2 to 46 h after death. Intensity and extension of staining were studied in ten regions. The staining gradually progressed after death also within non exposed areas. It most often started anteriorly on the tarsus and finally included the fornix.

Microscopy revealed diffuse cell staining by rose bengal or trypan blue. The cell nucleus was most often more intensely stained than the cytoplasm. Up to five h after death neutral red had only stained vacuoles in the cytoplasm. Later diffuse staining of cells occurred.

Tetrazolium differed from the above dyes in that the pronounced staining seen immediately after death gradually decreased in the course of time. Microscopy disclosed stained inclusion bodies in the cytoplasm up to 19 h after death. Then cell staining was only seen as a rare exception in relation to extracellular dye granules. However the mucous thread in the inferior fornix showed gradually increasing postmortal tetrazolium staining. The amount of mucus was found to be the same in dead persons as in the living.

The characteristic appearance of the dead eye is due among other things to ruptures of the corneal epithelium (fluorescein stained) and cell death and not to drying up or coating by mucus.

**Key words:** cornea - conjunctiva - vital staining postmortal double rose bengal - trypan blue - neutral red - fluorescein - tetrazolium isodonitrotetrazolium - alcian blue

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heading of this paper Postmortal Vital Staining may seem self contradictory. Strictly speaking we cannot *vital stain dead* persons. However the body takes longer to die than the individual as such and cell death extends to different regions at different intervals from the moment of death. The eyes change in appearance after death. They become glazed. Film producers wanting to give a perfect illusion of death can do so by instilling kerosene into the conjunctival sac. The object of the present study has been to clarify the superficial changes determining the postmortal appearance. Are they due to mucous coatings, desquamation of corneal epithelium, drying up or putrefaction? Where and when do the changes start? The eyes were vital stained and studied in slit lamp and microscope at different postmortal stages. The technique employed approximated that used in previous examinations of living eyes (Norn 1974).

### Method

Prior to vital staining I noted down whether the dead person's eye was closed or open and if open the width of the opening in millimetres. Then the eye was further opened and three dye drops of 0.01 ml each were tilted on the corneal centre as well as superiorly and inferiorly on the globe. The dye drop now flowed into the superior and the inferior fornix. The dye was thereafter distributed by blinking, carried out by the examiner moving the lids several times. Finally the eye was washed with 2-3 ml of a balanced saline solution (4.9 mg NaCl, 0.75 mg KCl, 0.48 mg CaCl<sub>2</sub>, 0.3 mg MgCl<sub>2</sub> in 1 ml). The following dyes were employed: A mixture of 1% fluorescein and 1% eosin or 1% bengal or a mixture of 1% iodonitrotetrazolium and 1/4% alcian blue or 1% trypan blue or 1% neutral red (Norn 1974). The vital staining was studied in Kowa's hand held slit lamp magnifying 10 times. After staining with tetrazolium five min were allowed to elapse before examination in order to ensure enzyme reaction. The results were graded from 1 to 5 (1 indicating only few dots (<30), 2 <100, 3 <1000, 4 <10000 and 5 >10000 dots). The results for the ten examined regions of each eye were entered in a diagram (Fig. 1). Specimens for microscopy were prepared by scraping with a platinum spatula from conjunctiva or cornea or by transferring a mucous thread from the inferior fornix to a glass slide between two small wooden sticks. Each specimen was kept in a moist chamber. For microscopy the glass slide was

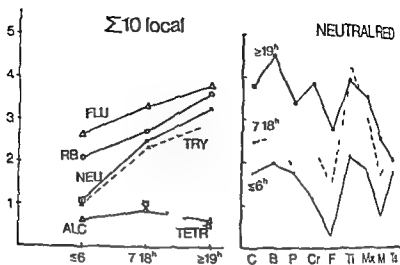


Fig 1

Left diagram Mean staining grade of ten regions at different intervals after death. FLU (fluorescein) assessed on the cornea only RB (rose bengal) NEU (neutral red) TRY (trypan blue) ALC (alcian blue) TETR (tetrazolium)

Right diagram Mean staining grade of nine regions at different intervals after death. C (cornea) B (bulb) P (plica) Cr (caruncle) F (inferior fornix) Ti (inferior tarsus) Mx (Mars line) M (mucous thread) Ts (superior tarsus)

covered by a cover slip holding one drop of balanced saline solution. Microscopy of the specimens stained by tetrazolium and neutral red for which time interval was critical followed within one h. The other specimens tolerated storing for up to 24 h.

The statistical calculation was performed using Mann Whitney's rank sum test for unpaired data.

### Material

A total of 202 eyes from 112 individuals with no diagnosed eye disease were examined. Mean age 72 (ranging from 36 to 91) median 73 (10 and 90 percentile 57 and 86 years) 47 per cent were females.

The dead bodies were kept in cellars (temperature about 10–14°C) and examined before being transferred to the postmortem room.

Vital staining was performed from 2 to 46 h after death in 45 cases, 6 h in 80 between 1 and 18 h and in 77 after not less than 19 h.

Microscopy was undertaken of 108 scrapings and 26 mucous threads from 57 individuals equally distributed over the different vital staining groups.

## Results

The eyes of normal living persons only become stained to a very small extent by the vital stains employed (Norm 1972a 1974). As a general rule the vital staining was found to increase in intensity and extension the longer the interval after death. Exceptions were tetrazolium and alcian blue (Fig. 1 left diagram and Table 1). The staining was independent of age and sex.

## Fluorescein

Corneal epithelial defects being therefore rarely seen in normal eyes.

The first three postmortal hours a moderate punctate staining was noticed spread over the whole cornea (on an average grade 1.8) but after four hours a more intense punctate staining was seen to cover the cornea (grade 3.5  $P < 0.05$ ). After 10 hours the great majority and after 20 hours the total number of eyes also displayed an extensively stained erosion. This was located centrally, being often transversely oval and comprising up to one third of the corneal area.

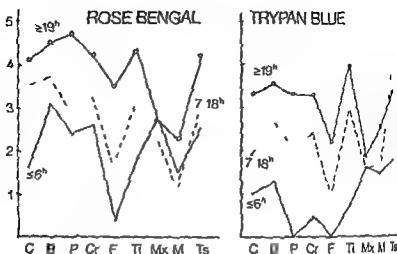
Increasing punctate staining was also seen on the conjunctiva.

The fluorescein staining and the erosion were independent of whether the eye had been open or closed postmortally.

Table 1

Postmortal vital staining. Mean staining grades at short and longer intervals after death assessed for five regions (h: hours after death). N: number of eyes. tarsus: inferior tarsus. n.s.: non significant.

Vital stain	h	N	Cornea	Bulb	Fornix	Tarsus	Mucous thread
Neutral red	≤ 6	19	1.75	2.00	0.95	2.17	1.08
	> 6	24	3.21	3.83	0.95	4.17	2.18
	P		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Rose bengal	≤ 6	8	1.63	3.13	0.38	1.75	1.50
	> 6	34	3.76	4.02	2.47	3.62	1.59
	P		< 0.01	< 0.05	< 0.01	< 0.01	n.s.
Trypan blue	≤ 6	12	1.00	1.33	0.00	0.75	1.50
	> 6	50	2.66	3.26	1.54	3.60	1.96
	P		< 0.01	< 0.01	< 0.01	< 0.01	< 0.05
Tetrazolium	≤ 18	41	1.56	1.15	0.63	1.32	0.88
	> 18	21	0.86	0.24	0.10	0.14	0.14
	P		< 0.05	< 0.05	< 0.05	< 0.01	< 0.01



Postmortal vital staining

Fig 2

Left diagram Postmortal vital staining of nine regions with rose bengal

Right diagram Vital staining with trypan blue

Abscissa regions abbreviated as in Fig 1 right

### Trypan blue

stains dead cells

The staining was modest within the first six h after death. It was most pronounced over the areas of the prekeratinized line along the lid (Mx and Cr) and the mucous thread which both contain many dead cells. This is also the case in living persons (Norn 1967).

The staining increased during the next h so much so that after more than 19 h all regions were stained to a degree no less than Marx line (right diagram Fig 2).

Soon after death the cornea showed punctate staining forming a horizontal band. Several h later such staining most often covered the whole cornea.

Soon after death the bulb was stained at about 3 and 9 o'clock and later also at about 6 o'clock. After 19 h the staining extended all the way round the limbus corneae.

Staining of the tarsus most often started anteriorly at the margin. It subsequently posteriorly (20 against 9 cases). Late examination showed the staining to have spread over the whole tarsus in most cases.

The staining was independent of whether the eye was opened or closed.

**Microscopy** Scraping and mucous threads showed zones with stained cells whose nuclei were more intensely stained than their cytoplasm.

### *rosc bengal*

ns degenerate cells death cells and mucus In normal eyes of living persons y Marx line caruncle and mucous thread become intensely stained whereas other regions are only slightly stained (Norn 1974)

The staining was seen to increase gradually in all regions after death reach a maximum everywhere by the late postmortem examination (left diagram 2 Table I) The staining seemed generally to be more extensive than er trypan blue

The staining was not like keratoconjunctivitis sicca staining localized within exposed area and it was independent of any closing defect On early imination universally spread punctate staining was seen over the cornea ending to the bulb above and below the cornea but rarely temporally nasally The triangular exposed area might even remain unstained unlike surrounding areas More than 18 h after death all the examined eyes owed dense punctate staining of most of the cornea and bulbar conjunctiva nerally round the entire limbus) spreading to the fornix and the tarsus e staining of the superior tarsus most often started anteriorly

*microscopy* Most of the cells from scrapings and mucous thread presented universally ead staining in some instances nuclei more so than cytoplasm

### *utral red*

ins inclusion bodies in the cytoplasm of the cells of living persons (Marner Norn 1978)

Slit lamp examination revealed a gradually increasing staining the longer e interval after death The grade and distribution of the staining corre onded approximately to that for trypan blue (Fig 1) The staining grade as less than that obtained with rosc bengal The staining independent of an en or closed eye most often started anteriorly on the tarsus

*microscopy* The first four h after death red staining was only observed in inclusion dies in the cytoplasm the same as in living persons From five h after death I also ticed weak diffuse staining of odd cells the same grade in nucleus and cytoplasm ter six h increasing diffuse cell staining occurred with the result that inclusion dies were no longer discernible After seven h the staining of nuclei was more in use than that of cytoplasm The number of stained cells increased After 18 h in usely stained regions were seen in which practically all the cells were involved - ist as in a well stained specimen prepared for histological examination

This development included both granulocytes and all species of epithelial cells The vital staining observed within the first five h after death represented a reaction living cells (stained inclusion bodies) After seven h all the stained cells were dead nes

This microscopical finding is in keeping with the fact that neutral red stains to a greater extent than trypan blue within the first six postmortal h while subequally the staining grade corresponds to that of trypan blue (compare right diagram F with right diagram Fig 2 neutral red stains 86% trypan blue 59% of 170 local  $< 6$  h  $t = 4.3$   $P < 0.001$ )

### Tetrazolium

rarely stains normal living eyes and if it does the staining is low grade punctate in the cornea in 9 per cent tarsus in 10 per cent Marx line in 5 per cent Norn 1963

As early as two to six h after death pronounced punctate staining was seen not only of the exposed cornea but also of the non exposed bulb tarsus and fornix. The staining of the cornea either formed a horizontal band or was diffusely spread. In rare cases there was intense staining of flakes or epithelial cells. The staining of the superior tarsus was most often spread over the whole tarsus but more rarely concentrated anteriorly and very rarely posteriorly along the tarsus. The colour faded the longer the interval from the moment of death. The reaction to tetrazolium thus contrasted with that to the above dyes. The inferior fornix was not stained after 21 h and the other areas not after 26 h.

The mucous thread in the inferior fornix was nearly always stained (93 per cent) and its red colour grew increasingly intense the longer the postmortal period in other words a reaction contrasting definitely with those of the other regions.

**Microscopy** Until 18 h after death red inclusion bodies were found in epithelial cells of scrapings and mucous thread. The stained cells were concentrated in zones. In the cornea often 50 to 80 per cent of the cells contained red inclusion bodies. The cells had absorbed the colourless tetrazolium and reduced this to red formazan in organelles of the cytoplasm (Norn 1974).

After 19 h no inclusion bodies were visible. Nests of red granules occurred extracellularly in the mucous thread. Occasional epithelial flakes having red granules. Such nests of dye granules were diffusely red with nuclei and cytoplasm equally stained. The pronounced local accumulation of formazan had evidently caused staining of cells as in a specimen for histological examination.

### Alcian blue

Alcian blue is a specific mucus staining dye. Mucus is always present in the living eye in the fornix. Punctate staining of mucus may in some instances also be seen on the bulbar conjunctiva the tarsus Marx line the caruncle and the cornea but more rarely the cornea (Norn 1963 1972b).

Fig 3 shows the mean staining grade in dead persons being recorded at different intervals after death. Punctate staining of mucus on the cornea was seen in 37 per cent and on the bulbar conjunctiva in 23 per cent not differing significantly from the living eye.

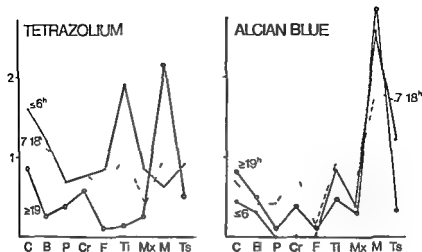


Fig 3

Postmortal staining with isodonitrotetrazolium (left diagram) and alcian blue (right diagram) Abscissa regions abbreviated as in Fig 1

A mucous thread is always present in the inferior fornix. Double staining with tetrazolium and alcian blue gave a mainly red mucous thread in 34 per cent suggesting bacterial infection (Norm 1974) and a mainly green one in the remaining cases.

**Microscopy** Extracellular mucous fibrils were seen in nearly all scrapings. In the mucous thread all the mucous fibrils had an intense green colour.

In a few exceptional cases odd green stained cells were seen from 9 h after death. After 18 h zones of such were present in 5 per cent where the nucleus was more intensely stained than the cytoplasm.

## Discussion

The results of the present investigation confirmed that cell death sets in later than the individual's death and that the cell death proceeds to different regions at different points of time after death. A kind of topography of death. The present investigation gave results suggesting that cell death most often starts anteriorly on the superior tarsus and reaches the fornix long after the other regions.

The glazed eyes (the altered appearances of cornea and bulbar conjunctiva after death) are hardly due to drying up the alterations being inde-



pendent of open or closed eye from the moment of death. Further the bengal stained area was not identical with the exposed one. On the cornea the exposed triangles of bulbar conjunctiva might remain unstained, while the surrounding area and the tarsus became intensely stained.

The postmortal changes were *not* due to deposits of mucus because amount of mucus does not increase after death and the mucous thread remains unchanged in the inferior fornix.

The glazed eyes were caused by increasing cell death (the cells were stained by trypan blue, neutral red and rose bengal) and corneal ruptures (puncta and erosions stained by fluorescein).

The time of death can roughly be estimated by vital staining, one eye with neutral red for instance and the other with tetrazolium and then studied with the result in slit lamp and microscope (Figs 1 and 3). Note however that considerable differences may be seen with regard to grade and extension of the staining between the two eyes of the same dead persons (Grade 1 in 11.7%, grade 2 in 3.3%, grade 3 in 0.3%, no difference in 84.7% - 1200 localities).

With neutral red the staining increases postmortally. The reverse is the case with tetrazolium; the latter only staining cells whose enzyme function is preserved. However, the mucous thread in the fornix shows increasing tetrazolium staining, evidently because extracellular detritus likewise may produce staining granules on an enzymatic basis.

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## THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

### III The Bulbar Zone the Zone of the Fornix and the Supranodular Zone

BY

STEFAN LATKOVIC

Three adjacent zones of the conjunctival epithelium of the guinea pig are described: the three layered bulbar zone with cuboidal superficial cells, the three layered zone of the fornix with cylindrical superficial cells, and the four to five layered supranodular zone characterized by close association with a lymphoid nodule. The similarities and differences in the general morphology of the epithelium and in the ultrastructural features of the cells as compared to those of the perilimbal zone are pointed out. The functional significance of atypical mitochondria found in certain cells of the bulbar and fornical zones is commented upon. Particular attention is paid to the supranodular zone containing a large number of intraepithelially located lymphocytes. The possible role of an epithelium associated with subepithelial lymphoid tissue in the immune defense is discussed.

**Key words:** conjunctival epithelium - bulbar zone - zone of the fornix - supranodular zone - atypical mitochondria - lymphocytes - guinea pig - ultrastructure - transmission electron microscopy

The morphology of the basal intermediate (Latkovic & Nilsson 1979a) and superficial (Latkovic & Nilsson 1979b) layers of the perilimbal zone of the guinea pig conjunctival epithelium was described in preceding papers. The present study concerns the ultrastructure of the bulbar zone, the zone of the

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pendent of open or closed eye from the moment of death. Further the bengal stained area was not identical with the exposed one. On the other hand the exposed triangles of bulbar conjunctiva might remain unstained, while the surrounding area and the tarsus became intensely stained.

The postmortal changes were *not* due to deposits of mucus because the amount of mucus does not increase after death and the mucous thread remains unchanged in the inferior fornix.

The glazed eyes were caused by increasing cell death (the cells were stained by trypan blue, neutral red and rose bengal) and corneal ruptures (punctures and erosions stained by fluorescein).

The time of death can roughly be estimated by vital staining one eye with neutral red for instance and the other with tetrazolium and then studying the result in slit lamp and microscope (Figs 1 and 3). Note however that considerable differences may be seen with regard to grade and extent of the staining between the two eyes of the same dead persons (Grade 1 a difference in 11.7%, grade 2 in 3.3%, grade 3 in 0.3%, no difference in 81% - 1200 localities).

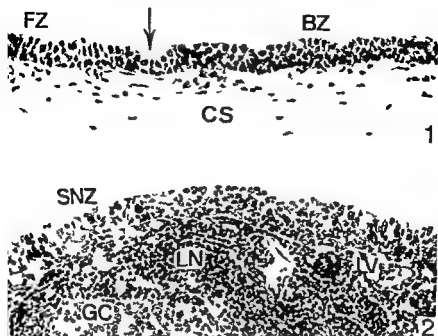
With neutral red the staining increases postmortally. The reverse is the case with tetrazolium; the latter only staining cells whose enzyme function is preserved. However the mucous thread in the fornix shows increasing tetrazolium staining evidently because extracellular detritus likewise may produce granules on an enzymatic basis.

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Figs 1 and 2

Low power light micrograph of the conjunctival epithelium showing the bulbar zone and the zone of the fornix (FZ). Note the rather uniform thickness of the epithelium. The conjunctival stroma (CS) contains only a few cells. To the right of the image are seen the cuboidal superficial cells of the bulbar zone. To the left of the image many of the cells are cylindrical which is typical of the zone of the fornix.

Low power light micrograph of the supranodular zone (SNZ) of the conjunctival epithelium. LN lymphoid nodule, GC germinal centre, LV lymph vessel  $\times 340$ .

in the corresponding cells of the perilimbal zone. Thus no dark and flat cells were found. The vesicular and vacuolar apparatus of the cells in intermediate and superficial layers was not as well developed and the intracellular cysts in the superficial cells were infrequent.

The microfilaments were less numerous in the intermediate and superficial layers. Melanosomes were absent throughout all layers. In some of the intermediate and superficial cells the common mitochondria were replaced for the most part by large and strangely shaped bodies presumably atypical mitochondria (Fig. 3).



## zone of the fornix

seen in survey light (Fig 1) and electron micrographs (Fig 4) the epithelium of the fornical zone was predominantly three layered. The basal cell layer was similar to that of the bulbar zone. The intermediate cell layer did not contain light and dark cells of the types found in the perilimbal zone but some polyhedral cells presumably transitional forms between the basal and superficial cells showed a less electron dense cytoplasmic matrix than the other ones accompanied by a decreased number of microfilaments and an increased number of vesicles and vacuoles.

As a marked difference between the bulbar and fornical zones the superficial cells of the latter zone were cylindrical in shape except at both peripheries of the zone where they were more cuboidal. The cytoplasmic matrix of the superficial cells was less electron dense than that of the basal and most intermediate cells and the microfilaments were fewer and more dispersed. Numerous mitochondria and a moderate number of vesicles and vacuoles were present. Large coalesced vacuoles were not observed however and the intracellular microfilaments were fewer than in the perilimbal zone.

Certain superficial and a limited number of intermediate cells in all other structural respects identical to other cells of the same layer contained a large number of strangely shaped bodies presumably atypical mitochondria (Figs 4 and 5). Very few common mitochondria were observed in these cells. The atypical mitochondria showed great variations in both shape and size including some rather bizarre forms. It seems that different planes of sectioning contributed to the great polymorphism seen in the micrographs. No serial sections were cut.

In the sections the atypical mitochondria hardly ever showed cristae of the lamellar type but instead profiles of what appeared to be saccular or tubular structures sometimes concentrically arranged. Many of them enclosed rounded segments (vacuoles) with a content similar to or slightly less electron dense than the cytoplasmic matrix. In several cases this segment was found to be continuous with the cytoplasm of the cell (Figs 4 and 5). These arrangements made some of the mitochondria appear ring shaped or crescent shaped respectively.

Fig 3

Electron micrograph of the intermediate polyhedral cells (P) and superficial cuboidal cells (S) of the epithelium of the bulbar zone. An intracellular cyst (CY) is demonstrated. Two of the superficial cells contain atypical mitochondria (AMI). N nucleus MI mitochondria I interdigitations MV microvilli  $\times 8750$ .



of the specialized cells a moderate number of goblet cells were observed in superficial layer increasing towards the supranodular zone of the epithelium (Fig 4). Small lymphocytes (Fig 4) occurred with moderate frequency and could be found in all cell layers of this zone. Plasma cells were rarely found and then only in the basal and intermediate cell layers.

#### **Supranodular zone**

A lymphoid nodule is located subepithelially near the base of the lid at about one-third of the distance between the limbus and the lid margin. It consisted of densely packed lymphoid cells with darkly stained nuclei except in the germinal centers where the cells had a lighter appearance (Figs 2 and 6). The population of lymphocytes was very heterogeneous showing morphological variations from small to medium sized lymphocytes with numerous intermediate forms. Isolated plasma cells and reticular cells were also seen. Small lymph vessels were distributed along the periphery and a few inside the nodule.

The overlying conjunctival epithelium was separated from the lymphoid nodule by the basal lamina (Figs 6 and 7). In light micrographs of sections stained with silver nitrate the basal lamina appeared to be discontinuous. Electron micrographs showed that at the points where the basal lamina was absent groups of lymphocytes were traversing it (Fig 7).

Inside the epithelium the lymphocytes were most often observed as small groups located in all layers (Figs 6 and 8). The cell membrane was smooth and no junctions between lymphocytes or between lymphocytes and adjacent epithelial cells were seen (Fig 8). The intraepithelial lymphocytes were predominantly of the medium sized type with variations mainly in the nuclear to cytoplasmic ratio and the quantity of cytoplasmic organelles. The shape of the nucleus followed the shape of the cell (Fig 8). Single ribosomes and polyribosomes were dispersed throughout the cytoplasm. The large Golgi complex was

**Fig 4**

Survey electron micrograph of the epithelium of the zone of the fornix. Above a layer of basal cells (B) a single intermediate layer of polyhedral cells (P) is seen. The superficial layer consists of cylindrical cells (CL) one of which contains a great number of atypical mitochondria (AMI). A goblet cell (GO) is interposed between the cylindrical cells. A small lymphocyte (LY) is located in the intermediate layer. N nucleus, MI mitochondria, VA vacuole, I interdigitations, F bundles of microfilaments. Arrow points to the basal lamina.  $\times 6000$ .





Fig. 2

Atypical mitochondria with cristae in form of tubules or saccules in a typical of the zone of the fornix. Some of the profiles are ring shaped or encircling cytoplasm with ribosomes and vesicles. Arrows point to the plane where the surrounded part of the cytoplasm is continuous with the cytoplasm of the mitochondria. VE small vesicles, VA vacuole, R ribosomes, N nucleus.  $\times 37,600$ .

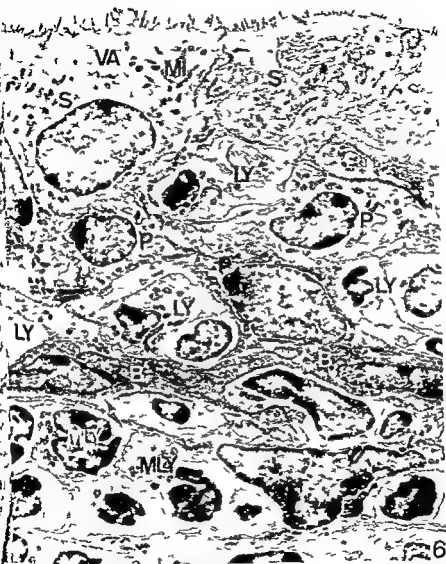


Fig 6

every electron micrograph of the epithelium of the supranodular zone and a part of the lymphoid nodule with lymphocytes some of which can be identified as medium sized lymphocytes (MLY). Three intraepithelial islets of aggregated lymphocytes (LY) are seen. The basal cells (B) appear flattened and the polyhedral cells (P) of the intermediate layers are rather irregular in shape. The microvilli at the free surface of the superficial cells (S) are uneven and sometimes strangely shaped. Arrow points to the basal lamina. VA vacuole MI mitochondria  $\times 3500$ .



Fig 7

A group of lymphocytes (LY) is seen partly in the lymphoid nodule and partly in the epithelium. The basal lamina is absent at this point. A polyhedral cell (P) and an intermediate layer is separated from a basal cell (B) by a lymphocyte (LY). A point in the basal lamina  $\times 13,100$

s vacuoles mitochondria some multivesicular bodies and long profiles of rough surfaced endoplasmic reticulum were usually found concentrated in certain section of the cell compartment. Structures resembling secondary lysosomes were observed in some lymphocytes.

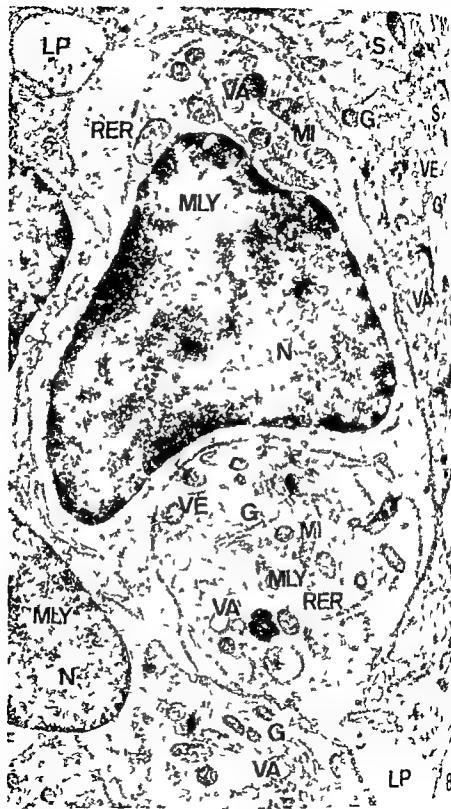
The conjunctival epithelium overlying the lymphoid nodule consisted of four to five distorted cell layers. The irregularities in the arrangement of the cells and in the shape of many of the cells was caused by the islets of invading lymphocytes. The basal cells were flatter than those of the previously described zones. As to ultrastructural aspects the basal cells and the polyhedral cells in intermediate layers resembled the corresponding cells in the fornical zone. Melanocytes and no melanosomes were observed. The superficial cells contained a moderate number of microfilaments. Large compound vacuoles and acellular cysts were absent. On the free cell surface the microvilli showed great variations in shape and incidence. In other respects the cells in the superficial layer were similar to those of the perilimbal zone.

Very few goblet cells were observed and only at the periphery of the supranodular zone.

### DISCUSSION

Many of the general ultrastructural characteristics such as the presence and distribution of intracellular components described and discussed for the three main epithelial cell types (basal intermediate and superficial cells) of the perilimbal zone of the conjunctival epithelium of the guinea pig (Latkovic & Nilsen 1979a, b) were also typical for the three zones of the epithelium covering the bulb, the fornix and the lymphoid nodule. Differences seen even at low magnification concerned the number of cell layers (three in the bulbar zone and zone of the fornix and four to five in the perilimbal and supranodular zones), the cell shape (cylindrical cells in the superficial layer of the zone of the fornix) and the cell types (melanocytes were absent whereas small numbers of goblet cells were observed in all zones described in the present paper). Certain cells in the superficial layer and less often in the intermediate layer of the bulbar zone and the zone of the fornix contained a large number of what appeared to be atypical mitochondria. Such atypical mitochondria were not observed in the other zones of the conjunctival epithelium.

As seen in the micrographs profiles of several atypical mitochondria resembled partly open rings. Similar structures in the interstitial cells of rat testis (Christensen & Chapman 1959) and in the cells of rat liver (Stephens & Bilson 1965) were previously described under the name of cup shaped mitochondria. In both reports the authors pointed out the increased contact surface between



mitochondrion and the cytoplasm facilitating the exchange of metabolic mediates as well as the possibility that the enclosed cytoplasm may be modified enzymatically. Cup shaped mitochondria were also observed by Rosen et al (1969) in alveolar cells of oxygen adapted and poisoned rats. The authors suggested that the increased surface to volume ratio might represent a compensatory response to diminished enzymatic activity. Similar mitochondria are reported for the lutein cells of the rat corpus luteum actively secreting progesterone (Enders & Lyons 1964). Other forms of atypical mitochondria observed in the adrenal cortex of hamster (De Robertis & Sabatini 1958 & 1965) mouse (Zelander 1959) rat (Sharawy & Penney 1973) and in renocortical cells in culture (Suyama et al 1971). Many of these authors regard the atypical mitochondria as one of the signs of active steroidogenesis. The question as to what the functional significance of the atypical mitochondria in the conjunctival epithelium might be and why they occur only in certain cells of the bulbar and fornical zone and not in any other parts of the epithelium cannot be answered at the moment.

The anatomical arrangement of an epithelium overlying a lymphoid tissue is described earlier for the intestinal tract (appendix Peyer's patches in the small intestine) and for the oropharynx (lingual palatine pharyngeal tonsils) (e.g. Rhodin 1974). Subepithelial aggregations of lymphocytes in the human conjunctiva some of which resemble lymphoid nodules were reported among by Norn (1960) and by Kessing (1968). The only mention of the subconjunctival lymphoid tissue in the guinea pig to our knowledge was made by Master et al (1967) and Tenner et al (1971).

Characteristic for the association of an epithelium and lymphoid tissue is the finding of a great number of intraepithelial lymphocytes aggregated in small groups. In the cases of the oropharyngeal and intestinal tracts it is generally considered that the lymphocytes migrate from the lymphoid tissue into the overlying epithelium. The basal lamina and the intercellular junctional complexes apparently do not hinder the movements of the lymphocytes through the epithelium. According to Steer (1975) the tissue boundaries are no barrier to the

*Fig. 8*

medium sized lymphocytes (MLY) located near the free surface of the superficial cells (S). Intracellular organelles such as mitochondria (MI), Golgi complex (G), vesicles (VE), vacuoles (VA) and profiles of rough surfaced endoplasmic reticulum (ER) are concentrated at one pole of the lymphocyte. The nucleus (N) is located at opposite pole. The cell membrane is smooth without interdigitations and intercellular junctional complexes. LP lymphocyte process, MV microvilli.  $\times 16,300$ .

migration of the cells from the tissue of mesodermal origin through the endodermal origin. The fate of the intraepithelial lymphocytes has not yet been elucidated. Some authors (Faulk et al 1971, Rhodin 1974, 1975) favour a theory that they leave the epithelium via the free surface and others (Mcader & Landers 1967, Glaister 1973, Bjerregaard 1975) say that the lymphocytes return to the subepithelial lymphoid tissue. The rather low number of lymphocytes found in human tear fluid (Norn 1960) supports the former theory. In our material we could not find decisive signs supporting one of these theories.

While the lymphoid nodule contained a heterogeneous population of lymphocytic cells, the lymphocytes observed inside the epithelium all had some common characteristics, also indicating an active cell: a large Golgi complex, an increased number of mitochondria and of free ribosomes, polyribosomes and profiles of rough surfaced endoplasmic reticulum. In the lymphoid tissue these cells are known as "intermediate" or medium sized lymphocytes (Zucker-Franklin 1969, Rhodin 1974).

Similar cells were found in the bursa of Fabricius (Clawson et al 1970), Peyer's patches of rabbit intestine (Faulk et al 1971), in the intestinal epithelium of the mouse (Glaister 1973) and in the intestinal epithelium of the chicken (Bjerregaard 1975). It was suggested by the above mentioned authors as well as by Fichtelius (1967) that intraepithelial lymphocytes and subepithelial lymphoid tissue participate in immune responses. According to Bjerregaard (1975) an immunologically competent small lymphocyte can be stimulated by antigen and transported through the epithelial cells. Depending on the nature of the antigen, either cellular immunity is triggered or the stimulated cell differentiates into an IgA synthesizing plasma cell. In the course of this process the small lymphocyte passes through stages in which it is indistinguishable from the intermediate lymphocyte (Rhodin 1974).

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# THE ULTRASTRUCTURE OF THE NORMAL CONJUNCTIVAL EPITHELIUM OF THE GUINEA PIG

## IV The Palpebral and the Perimarginal Zones

BY

STEFAN LATKOVIC

*The ultrastructure of the palpebral and perimarginal zones is described and compared to that of the other zones of the conjunctival epithelium of the guinea pig. The palpebral zone was characterized by frequently occurring goblet cells the structure and function of which are discussed. The transition in the perimarginal zone from a four layered epithelium with cylindrical superficial cells in the palpebral zone to a multilayered squamous epithelium towards the lid margin is shown.*

*Key words:* conjunctival epithelium - palpebral zone - perimarginal zone - goblet cell - guinea pig - ultrastructure - transmission electron microscopy

The present description of the palpebral and perimarginal zones concludes a series of papers concerning the ultrastructural survey of the normal conjunctival epithelium of the guinea pig. The preceding papers described the perilimbal zone (Latkovic & Nilsson 1979a,b), the bulbar zone, the zone of the fornix and the supranodular zone (Latkovic 1979). The aim of the study was also to provide a background for an investigation of phagocytosis in conjunctival epithelial cells (Latkovic & Nilsson 1979c). The palpebral zone, comprising the longest part of the conjunctival epithelium, begins at the distal limit of the supranodular zone and merges into the perimarginal zone, which in turn constitutes the zone of gradual transition from

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the conjunctival epithelium to the keratinized epidermis at the lid margin, the palpebral zone and to some extent also the perimarginal zone included in the survey of the basic cell types described in the preceding papers (Latkovic & Nilsson 1979a, b). The present paper therefore concentrates on the morphological differences between the zones described here and those reported on earlier.

Previous electron microscopic studies of the palpebral/tarsal conjunctiva concern human material. Greiner et al. (1977) described the surface morphology of the conjunctiva as seen in scanning electron microscopy. Dark et al. (1977) focused on histochemical and transport problems. Both studies concerned superficial cells and were discussed in detail in the paper on the superficial layer of the perilimbal zone (Latkovic & Nilsson 1979b). Takakusaki (1977) described the basic cell types of human tarsal conjunctiva and the changes in vernal conjunctivitis. A picture of the tarsal conjunctiva was published in a paper by Weingest (1973a). The perimarginal zone was described briefly in a survey of vertebrate epithelia (Parakkal & Alexander 1977).

## Material and Methods

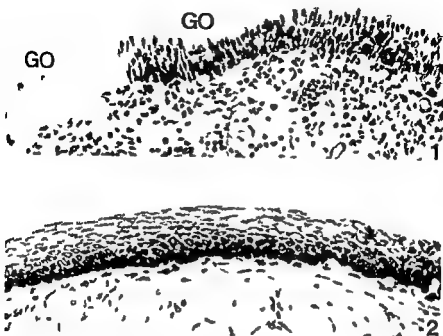
Conjunctival specimens from four clinically healthy pigmented guinea pigs were prepared for light and electron microscopy according to the methods described in detail in the preceding papers (Latkovic & Nilsson 1979a, b).

## Observations

### The palpebral zone

The palpebral zone was the longest part of the guinea pig conjunctiva, and its general appearance is seen in Fig. 1 which is a light micrograph. The epithelium showed a rather uniform configuration with minor variations concerning the number of cell layers and incidence of goblet cells. There was a rather distinct border towards the epithelium of the supranodular zone while at the opposite end the transition to the epithelium of the perimarginal zone was gradual.

The ultrastructural characteristics of the epithelium of the palpebral zone are demonstrated in a survey electron micrograph (Fig. 3). The basal layer was composed of basal cells and occasional small lymphocytes (Fig. 3). Melanocytes and melanosomes were absent. The intermediate layer generally contained up to two layers of polyhedral cells. These cell types were similar to the corresponding ones in the perilimbal zone. Immature goblet cells at different stages of development were also observed in the intermediate layer (Fig. 3). In addition, with a small number of mucous globules the latter were distributed randomly.

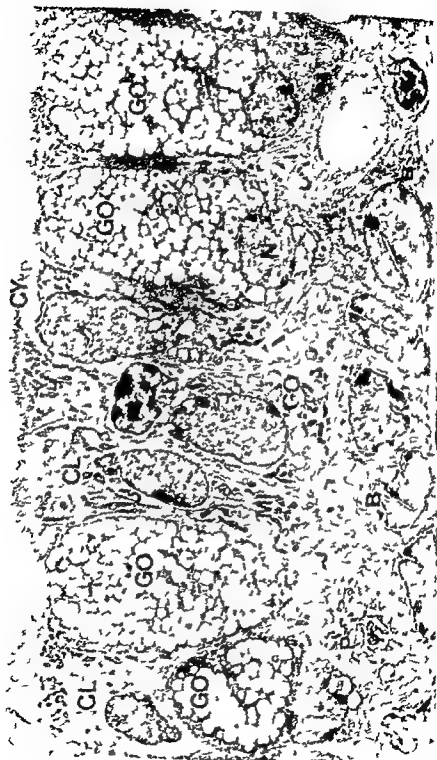


Figs 1 and 2

Survey light micrograph of the conjunctival epithelium of the palpebral zone. The epithelium consists of 3-4 layers. Lightly stained goblet cells (GO) in the superficial cell layer are seen either solitary interspersed between the cylindrical cells or in rows. The conjunctival stroma contains a large number of cells.  $\times 340$

Survey light micrograph of the epithelium of the perimarginal zone. The transition from cuboidal cells (to the right) to squamous cells (to the left) is demonstrated as an increasing number of cell layers. The dark appearance of the cells in the basal and subbasal layers is due to the presence of melanin. The nuclei of the cells constituting the outer half of the squamous epithelium are very lightly stained. The conjunctival stroma is characterised by a paucity of cellular elements.  $\times 300$

the cytoplasm while the nucleus was large and located centrally. With increasing mucous content the cell assumed an elongated shape with the upper half compartment packed with mucous globules, the nucleus displaced to the lower half of the cell and the amount of cytoplasmic matrix and the number of intracellular organelles correspondingly decreased (Fig. 3). The superficial cell layer consisted of cylindrical epithelial cells and mature goblet cells often in groups alternating in an irregular way (Fig. 3). Both cell types were greatly elongated and sometimes even represented more than half



epithelial thickness. In such a case the intermediate cell layer could be bent (Fig. 3). The cylindrical cells did not differ from those of the zone of fornix. They contained vesicles, vacuoles and intracellular cysts (Fig. 3) of the same kind as observed in the superficial cells of the perilimbal and bulbar zones and the zone of the fornix. The free cell surface was covered with microvilli. Towards the perimarginal zone the shape of the superficial cells was more oval.

The mature goblet cell was almost entirely occupied by tightly packed mucous granules, single or coalesced and sometimes seen to be membrane bound (Figs. 3 and 4). Some globules showed a single electron dense core or granule. The spaces between the globules were filled with electron dense granulated material in which ribosomes and occasional mitochondria could be identified (Fig. 4). At the free surface the goblet cell was either covered by a thin layer of cytoplasm and a cell membrane with microvilli or it could be open (Figs. 3 and 5).

The tightly interdigitating cytoplasmic processes and the intercellular junctions of the cylindrical and goblet cells were similar to those of the superficial cells of the previously described zones.

Small lymphocytes were the only migrating cells found in the palpebral zone and they were observed in all cell layers (Fig. 3).

#### perimarginal zone

In the zone of transitional epithelium the perimarginal zone is interposed between the palpebral conjunctiva and the lid epidermis. Adjacent to the palpebral zone it resembled the conjunctival epithelium and at the lid margin it was similar to the epidermis. The middle part consisted of a multilayered squamous non-keratinized epithelium.

A survey electron micrograph (Fig. 6) shows the basal and intermediate cell layers of a section from an area not too far from the palpebral zone. The basal layer with basal cells and melanocytes closely resembled the corresponding

*Fig. 3*

A low power survey electron micrograph of the conjunctival epithelium of the palpebral zone. The superficial layer consists of cylindrical cells (CL) and rather numerous goblet cells (GO). An intracellular cyst (CY) is seen in a superficial cell. Immature goblet cells are found in the intermediate layer which is rather indistinct in this figure. Small lymphocytes (LY) are present in the basal and superficial layers. B: basal cell, P: polyhedral cell in the intermediate layer  $\times 3950$ .

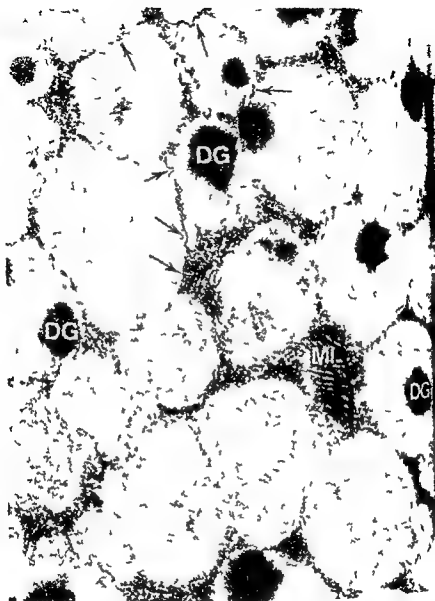


Fig. 1

Electron micrograph of mucous globules from a goblet cell. Enclosing membranes can be observed in certain places (arrows). Some globules appear partly coalesced. A variation in electron density between different globules is noticed and sometimes an electron dense granule (DG) is present. Mitochondria (MI) and ribosomes are also visible in the dense granular material filling the interglobular spaces.  $\times 33,000$ .



Fig 5

anning electron micrograph of a group of goblet cells (GO) more or less open at the surface CL cylindrical cells Arrows point to the intercellular borders  $\times 4000$

of the perilimbal zone The presence of melanin (Fig 6) caused the dark  
 range of the cytoplasm of these cells observed also in light micrographs  
 ig 9) The suprabasal and inner intermediate cell layers were composed of  
 per flat polyhedral cells with a moderate quantity of cytoplasmic organelles





7 6) Transversely cut dendritic processes with a smooth cell membrane and presence of intercellular junctions were observed between the basal and polyhedral cells (Fig 6) They could be identified as melanocyte extensions only when containing melanosomes (Fig 6) As the sections were cut farther away from the palpebral zone the cells of the outermost intermediate layers were found to be much flatter (Fig 7) The number of microfilament bundles was decreased What appeared to be partly degraded mitochondria were observed in the cells (Fig 7) The cytoplasmic processes were shorter in the outer layers The superficial cells showed a cytoplasm with few microfilaments some vesicles and ribosomes and a free surface covered by microvilli (Fig 7) In the periphery of the perimarginal zone the epithelium had the configuration of a multilayered nonkeratinizing squamous epithelium (Fig 8) The number of cell layers was further increased primarily due to numerous layers of very flat squamous cells in the outer part of the epithelium as compared to the area presented by Figs 6 and 7 The nucleus was flat and lightly stained The cytoplasm contained a moderate number of fibrillar strands some vacuoles and a large number of dense particles (Fig 9) Desmosomes occurred rather frequently (Fig 9) The microvilli were absent on the free surface of the superficial cells The inner part of the epithelium consisted of a rather large number of polyhedral cell layers (Fig 8) and a single basal cell layer The majority of the nuclei contained minimal quantities of chromatin and appeared less dense than the cytoplasm both in light (Fig 2) and in electron micrographs (Fig 8) Fibrillar material was present in somewhat greater amounts than in the cells of the outer layers The mitochondria were few and often appeared as vacuoles with residues of cristae (not shown) Other cytoplasmic organelles were observed only rarely

*Fig. 6*

the basal and deeper intermediate cell layers of the perimarginal zone from a section cut too far from the palpebral zone The intermediate cell layers are numerous The polyhedral cells (P) in the deeper intermediate layers appear almost identical in shape and cytoplasmic content Dendritic processes (DP) are observed in the basal as well as in the intermediate layers When such processes contain melanosomes they can be identified as melanocyte processes (MP) Most of the melanosomes (M) are found in the basal layer concentrated in melanocytes (MC) but also to a less extent in basal cells (B)  $\times 6900$



## Discussion

The palpebral zone of the guinea pig conjunctival epithelium showed general conformity to the palpebral/tarsal zone of the human conjunctiva as described by Takakusaki (1969) Weingeist (1973a) Dark et al (1974) and Greiner et al (1977). The main difference was the shape of the superficial cells which was cuboidal in the human (Takakusaki 1969 Weingeist 1973a Dark et al 1974) whereas in the guinea pig it was mainly cylindrical. However at the periphery of the palpebral zone the cell shape was more cuboidal even in the guinea pig epithelium. Takakusaki (1969) reported an increased number of filaments in the superficial cells which was not observed in our material.

Characteristic for the conjunctival epithelium of the palpebral zone of the guinea pig eyelid was the large incidence of goblet cells particularly in the part adjacent to the supranodular zone. It should be pointed out that the present study is based on thin sections cut in the meridians along the middle of the eyelid and that the distribution of goblet cells along other meridians might be different as was demonstrated for human conjunctiva by Hessing (1969). In the present material goblet cells were not found in the basal cell layer. Immature goblet cells were first observed in the suprabasal cell layer. In studies on the origin of goblet cells in the small intestine Merzel & Leblond (1969) Cairnie (1970) and Cheng (1974) concluded that immature mucous cells derive from the transformed columnar epithelial cells and that the number of such immature mucous (goblet) cells further increased by division.

The goblet cells in the conjunctival epithelium of the guinea pig were similar to the goblet cells observed in the human conjunctiva (Wanko et al 1964 Takakusaki 1969 Radnot 1971 Weingeist 1973b) and throughout the epithelial lining of the respiratory and digestive tracts (Freeman 1966 Cheng 1974 Rhoads et al 1974). Two subgroups of goblet cells, one containing mucous globules with dense granules and another without such granules, were observed in mouse small intestine (Cheng 1974) and also in our material. The granular and non-

Fig. 1

superficial (S) and outer intermediate cell layers of the perimarginal zone from a section closer to the palpebral zone than to the lid margin. The cells are rather flat and the cytoplasm is seen to contain a number of larger vesicles (VE) which are concentrated in the apical part of the cell and short bundles of microfilaments (F). Nucleus (N), partly decomposed mitochondrion (MV), microvilli (D), desmosomes (D).

× 12 900



nular mucous globules might possibly represent sequential developmental stages

It is generally accepted that the mucus derived from the goblet cells in the conjunctiva forms a mucous coat on the surface of the corneal and conjunctival epithelium (Wolff 1946 Mishima 1965). Since the critical surface tension of mucus exceeds that of tear fluid a continuous tear film is securely held in place (Lemp et al 1970 Holly & Lemp 1971). Isolated mucous strands/particles on the surface of the conjunctival epithelium were shown by Pfister (1975) and Greiner et al (1977). The ultrastructure and enzyme activity of the mucous strands were described by Egeberg & Norn (1967) and Norn (1971). No mucous strands were seen in our material. Either it was dissolved during the preparation procedure or it was not demonstrable with the technique used. The filamentous projections from the free surface of the superficial cells observed in our material which are considered to be different from the mucus and an integral part of the cell membrane were discussed in a previous paper (Latkovic & Nilsson 1979b).

The transition from the conjunctival epithelium of the palpebral zone to the dermis at the lid margin which could be followed across the perimarginal zone was similar to that described by Tarakka & Alexander (1972). Certain layers of the epithelium of the perimarginal zone resemble other stratified squamous nonkeratinized epithelia such as the buccal and palatal epithelium (Lerman 1971) and the epithelium of the soft palate and of the oesophagus (Rodin 1974). Towards the lid margin the epithelium began to show certain changes that have been described as preceding keratinization (Brody 1968 Matsuyama 1976): decomposition of nucleus and cytoplasmic organelles, increased amount of filaments in the outer layers and absence of microvilli on the apical surface.

*Figs 8 and 9*

Survey electron micrograph of the epithelium at the proximity of the lid margin showing the large number of cell layers. The inner layers are composed of cells with polyhedral shape while the outer layers consist of very flat squamous cells. The amount of nuclear chromatin is minimal and most nuclei (N) are more electron lucent than the cytoplasm.  $\times 7700$ .

Electron micrograph of squamous cells in the outer layers of the perimarginal zone rather close to the lid margin. Fibrillar material (F) and dense particles are the main components of the cytoplasm. D: desmosomes.  $\times 8500$ .

## Acknowledgment

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## JUDICIA DE NOVIS LIBRIS

*Hans Sautter Wolfgang Straub & Hermann Rossmann Atlas of the Ocular & Photographs of Typical Changes in Ocular and Systemic Disease* L. Schwarzenberg München Wien Baltimore 1977 160 pages 371 coloured photographs (with single exceptions) and accompanying text and subject Price Dkr 457 75

The book is not only a photographic atlas but also a collection of ultra short histories as each photograph is accompanied by a brief explanatory text and many cases also contains clinical points of important nature

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*Ernst Gierke*

*Greve E. L. (ed) Medical Therapy in Glaucoma* W. Junk by Publishers The Hague 1977 187 pages Price approx 45 Dutch Gld

This is volume 19 in the Documenta Ophthalmologica Proceedings Series

The reported symposium on medical therapy in glaucoma took place in Amsterdam in 1976 The symposium dealt with cholinergic drugs including ocusert pilocarpine and adrenergic drugs and their effect in the treatment of glaucoma with mention of the  $\beta$  adrenoreceptor blockings drugs The final chapter of the book deals with the clinical aspects of the medical treatment of glaucoma P. Graham (Cambridge) discusses the very important criteria for initiation of treatment in patients with suspected glaucoma E. L. Greve (Amsterdam) discusses some aspects of the management of glaucoma patients Finally Redmond Smith (London) discusses the medical and the surgical therapy of glaucoma patients In a sensible and well balanced way this small book deals with aspects of the treatment of glaucoma and is valuable to and not least the practising ophthalmologists who are concerned with the treatment of this often difficult group of patients

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## ULTRASONOGRAPHIC STUDY ON CHANGES IN AXIAL EYE DIMENSIONS AFTER ENCIRCLING PROCEDURE IN RETINAL DETACHMENT SURGERY

BY

JON S. LARSEN and PER SYRDALEN

The pre and postoperative results of ultrasonographic measurements on the axial ocular components in 10 phakic eyes with retinal detachment treated with encircling silicone rubber band are presented. A significant increase ( $P < 0.001$ ) in axial eye length from 0.62 to 1.94 mm (average 0.93 mm) was found. The elongation of the eye was caused by a corresponding increase in the length of the vitreous cavity. No significant changes were found in the anterior segment of the eyes. These data demonstrate that the postoperative refractive change in a myopic direction which an encircling procedure with moderate indentation often produces is caused by an axial elongation of the eye.

*Key words:* ultrasonography – retinal detachment – encircling procedure – axial eye length

Ultrasonographic measurements on eyes with retinal detachment treated with an encircling procedure have shown that this procedure often changes the axial length. A shortening seems to be as usual as an elongation (Miettinen & Ursin 1969, Flament 1973, Burton et al 1977). A myopic shift in refractive power in our experience often produced by a moderate indenting equatorial encircling procedure. We have not observed changes in the parameters of the anterior segment. The myopic change has therefore been thought to be produced by an increase in axial length.

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The purpose of the present study was to demonstrate possible changes in ocular parameters (corneal radius of curvature anterior chamber depth lens thickness vitreous length and axial length) induced by this method of encircling procedure. This could clarify the cause of a postoperative change in refraction.

## Material and Methods

The material includes 10 patients (6 males 4 females average age 59 years (14-76)) with retinal detachment in 10 phakic eyes. All eyes were treated with an encircling silicone rubber band of 2 mm width (Laticron Company, S-2969) combined with small silicone rubber explants under the band in order to create the local buckling. In all cases cryopexy and drainage of vitreous fluid were performed. The band was tightened appropriately to make a moderate equatorial indentation.

The corneal radius of curvature was determined with the Javal-Schiotz keratometer (Haag-Streit) in the main meridians preoperatively and repeated postoperatively. The anterior chamber depth (including corneal thickness) lens thickness and vitreous length were measured ultrasonographically preoperatively and repeated 5 to 8 weeks postoperatively. An A-scan echograph (Technic model 7200 MA and a 10 MHz/5 mm transducer (DM 10 St. 1)) was used. The measuring technique is described in detail in a previous paper (Larsen 1979).

All measurements were performed under cycloplegia. An average of three measurements was obtained pre- and postoperatively of each eye. The ocular distances were expressed in millimeters using the following ultrasonic velocities: 1532 m/sec in the anterior chamber and vitreous and 1641 m/sec in the lens (Jansson & Kock 1962).

The reliability of the method was tested on one eye with retinal detachment. Five measurements were carried out in each of two separate sessions (A and B) and the data are listed in Table I. The maximal deviations from the mean values based on these 10 measurements were: anterior chamber depth 0.1 mm lens thickness 0.08 mm vitreous cavity length 0.18 mm and axial length 0.17 mm. Any difference between pre- and postoperative values less than these maximal deviations are in the following considered as real.

In order to obtain the total length of the optical system (axial length) the thickness of the retina (0.4 mm) was added to the calculated axial length (François & Goes 1971).

Refractive power could be determined preoperatively in seven eyes. Postoperative refractive changes are based on these cases.

Table I

Axial dimensions in the control eye measured in two separate sessions (A B)  
Mean values of 5 measurements each session

Axial eye dimensions	Series of measurements			
	A		B	
	Mean mm	SD	Mean mm	SD
Anterior chamber depth	3.61	0.03	3.67	0.03
Lens thickness	4.46	0.03	4.48	0.06
Vitreous length	18.83	0.11	18.81	0.11
Eye length	26.90	0.11	26.91	0.15

## Results

Table II shows the pre and postoperative mean values of the minimum and maximum meridional corneal powers. As evident from this table no important differences were found either between the average minimum (0.04 dptr) or between the maximum meridional corneal powers (0.20 dptr) generated by the encircling procedure. The postoperative power changes in spherical equivalents were between -0.5 dptr and +0.5 dptr in eight eyes and between 0.5 dptr and -1.25 dptr in two eyes.

Table III shows the pre and postoperative data on the axial eye length. The encircling procedure produced a significant elongation of the axial length in 11 eyes examined. The average elongation was 0.98 mm ( $\pm 0.20$  mm) with

Table II

Pre and postoperative corneal curvature in diopters

Major meridian power	Preoperative corneal curvature dptr		Postoperative corneal curvature dptr	
	Mean	SD	Mean	SD
Minimum	47.09	0.19	42.13	0.19
Maximum	49.93	0.75	43.13	1.07

Table III

Axial length in 10 phakic eyes before and 5 to 8 weeks after encircling operation

Patient No	Preoperative axial length (mm)	Postoperative axial length (mm)	Postoperative lengthening (mm)
1	23.22	24.25	1.03
2	23.40	24.02	0.62
3	23.53	24.15	0.62
4	23.67	24.91	1.24
5	23.71	24.69	0.98
6	24.11	25.06	0.95
7	24.17	25.00	0.83
8	24.69	25.58	0.89
9	24.84	25.73	0.89
10	25.42	26.61	1.19
Mean	24.08	25.06	0.95
SD	0.71	0.75	0.90

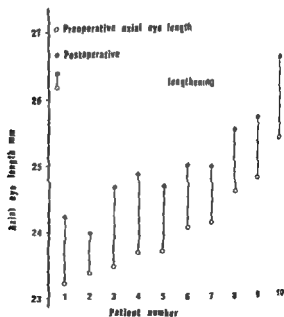


Fig. 1

Increase in axial eye length produced by encircling operation

Table IV

Anterior chamber depth and lens thickness before and 3 to 8 weeks after encircling procedure

Patient No	Anterior chamber depth mm		Lens thickness mm	
	Preop	Postop	Preop	Postop
1	3.92	3.91	3.55	3.59
2	3.49	3.41	4.66	4.67
3	3.54	3.53	4.15	4.13
4	3.63	3.67	4.43	4.47
5	3.11	3.07	4.86	4.86
6	3.13	3.11	4.55	4.36
	3.82	3.87	3.69	3.67
8	3.06	3.01	4.66	4.67
9	3.90	3.83	3.84	3.86
10	3.78	3.11	3.81	3.82
Mean	3.59	3.57	4.21	4.21
SD	0.31	0.32	0.46	0.46

extreme values between 0.62 mm and 1.24 mm (Fig. 1). The results showed that all eyes had a corresponding significant elongation of the vitreous cavity. The average increase of vitreous length was 1.00 mm ( $\pm 0.20$  mm) with extreme values between 0.62 mm and 1.26 mm. There was a positive correlation between the postoperative elongation of the axial lengths and the corresponding data for the vitreous lengths, the correlation coefficient being  $r = 0.997$ ,  $P < 0.001$ .

The pre- and postoperative data on the anterior chamber depth (included thickness of cornea) as well as on lens thickness are shown in Table IV. No essential differences in any of the individual measurements were found for these parameters. The average preoperative anterior chamber depth was 3.59 mm ( $\pm 0.32$  mm) compared to 3.57 ( $\pm 0.32$  mm) in the postoperative measurements. The average lens thickness was 4.21 mm ( $\pm 0.46$  mm) in the pre- and postoperative measurements. The mean difference between the pre- and postoperative refraction power was  $-2.4$  dptr ( $\pm 1.31$  dptr).

## DISCUSSION

Experimental encircling procedures with silicone bands of 3 mm width formed on eyebank eyes (IOP 20 mmHg) have shown that the axial length depends on the tightening of the band (Rubin 1953). According to his study a low, moderate and high indentation of the band (corresponding to a mean reduction in outer equatorial diameter of 0.42 mm, 0.93 mm and 2.1 mm) produced the following changes in the mean axial length: 0.44 mm, +1.09 mm and -0.35 mm. The average axial elongation of 0.93 mm from our study corresponds approximately to the elongation in axial length of 1.09 mm produced by a moderate band indentation obtained in Rubin's studies. These facts indicate that an encircling procedure with moderate indentation of the band will produce an elongation of the eye, thus inducing an essential refractive change.

The disparity in results found in studies on this topic (Miettinen & Törmä 1963; Flament 1973; Burton et al. 1977) is possibly caused by different surgical techniques and tightening of the encircling band. The surgical procedure must also have an influence on the axial dimensions of the anterior chamber. Contrary to our findings, which did not show any changes in the anterior segment dimensions (Table IV), other authors have found shallowing of the anterior chamber (Fiore & Newton 1970; Hartley & Marsh 1973; Burton et al. 1977) and increased lens thickness (Burton et al. 1977) after encircling procedures. Some authors concluded that the shallowing of the anterior chamber was transitory or possibly transitory (Fiore & Newton 1970). Our study included no cases with a postoperative choroidal detachment.

The refractive power of any eye depends both on the axial length and on the refractive power of the ocular components. We could not show any significant changes in the anterior segment dimensions (Table IV) or in the corneal refractive power (Table II). The myopia which regularly occurs must therefore be caused by the axial elongation. According to Rubin (1953) 1 mm axial increase produces 2.564 dpt of myopic shift at the spectacle lens plane in the phakic eye (the Gullstrand schematic eye). If this factor is used for the eyes in our investigation, the approximate values for the postoperative refractive changes can be estimated. According to this, the encircling procedure corresponds to changes in a myopic direction from -1.59 dpt (0.62 mm axial elongation) to -3.18 dpt (1.24 mm axial elongation). The average refractive change is -2.51 dpt (0.98 mm axial elongation). This corresponds in a high degree to the mean refractive change of -2.4 dpt found in our study. Myopic changes of this magnitude have also been shown by Jacklin (1971) and Rubin (1953). The present study has shown that the increase in axial length occurs regularly. This fact has to be considered when the operative procedure for a choroidal detachment is planned.

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# RETINOBLASTOMA IN SWEDEN 1958-1971

## A Clinical and Histopathological Study

BY

ERIK KOCK and PETER HAESER

This study includes all cases of retinoblastoma reported in Sweden between 1958 and 1971. The incidence of the disease was 1 per 15 000 live born. Only in six cases was there a familial history and five of these cases were bilateral. The tumour was bilateral in 3.5% of all cases. All cases of unilateral tumour had been treated with enucleation. In the bilateral case one eye had also been enucleated and the other eye treated by local irradiation therapy. Tumour invasion into choroid was found in 29% and into the optic nerve in 11% of the cases. The mortality was only 15%.

*Key words:* Retinoblastoma - clinic - histology

Retinoblastoma is the most common intraocular tumour in childhood. In the past years it has often been discussed whether there has been an increase in the incidence or manifestation of this malignant disease (Tahvanainen & Tuovinen 1971; Bishop & Madson 1975). It was therefore decided to study the clinical and histological aspects of all cases of retinoblastoma reported in Sweden over a 13-year period and to compare the results with those from other countries.

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## Material and Methods

The present material was obtained from the Swedish Cancer Registry (SCR) and comprised 94 patients. The data on six of the cases could not be traced in the clinics which had reported them to SCR or from the histopathological registration number of the cases. In the remaining 88 cases the medical records obtained from 28 different clinics were studied. Histological sections were obtained from 75 of the cases from 12 different pathological laboratories. The microscopic slides had been stained with either haematoxylin and eosin or van Gieson stain. Each section was studied by both authors independently with respect to 19 different variables. The mean value of each variable was expressed in per cent of the total number of cases studied.

## Results

## Clinical observations

The present material comprised 48 boys and 40 girls with retinoblastoma giving an incidence of 1 per 18 000 live births. The initial sign was a pupillary change in 51 cases. Squint was noted in 17 cases and acute red eye in 8 cases. Of the remaining 12 cases three were diagnosed on routine examination because of a familial history, four on clinical examination after head injury and four on routine clinical examination at child health centres. In only one case was information lacking about the presenting sign.

The duration of the symptoms before diagnosis can be seen in Table I. Approximately 50% displayed eye symptoms for less than 2 months. In one case a glittering pupil was noted by the parents for 2½ years.

Table I  
Duration of eye symptoms before tumour diagnosis

Duration of symptoms (months)	No. of cases
< 1	27
1 - 2	13
3 - 5	11
6 - 10	13
11 - 15	9
> 15	6

**Table II**  
Age of the 88 children at the time of diagnosis.

Age	No. of cases	
	Total	Bilateral
0 - 3 months	5	0
3 - 6 months	12	0
6 - 9 months	11	3
9 - 12 months	3	0
12 - 18 months	14	0
18 - 24 months	13	4
2 - 3 years	16	3
3 - 4 years	10	0
4 - 5 years	2	0
5 - 6 years	1	0
> 6 years	3	0
(6 ½ and 9 ½ years)		

There were 88 bilateral (18 boys and 15 girls) 31 right sided (15 boys and 16 girls) and 24 left sided (14 boys and 10 girls) cases. Twenty six of the bilateral cases were diagnosed at the first examination. During the first six months following unilateral diagnosis a tumour was observed in the second eye in two cases and during the next six months three more cases were found. In two cases did a tumour occur in the second eye more than 12 months later.

The age distribution of the children at the time of diagnosis can be seen in Table II. Two thirds of the cases were diagnosed within the first two years of life. After the age of four years only six cases were diagnosed. The majority of the bilateral cases (64 %) were discovered during the first year of life and in such case was found after the age of three years.

A familial history of retinoblastoma recorded in only six (five bilateral cases). The clinical records gave no information about familial history in the (three bilateral) cases.

Four patients died from the disease. The tumour was located in the retina in one, the left eye in one and bilaterally in two patients. In none of the patients was there a familial history. In two of these cases the initial signs were a pupillary change. Both of these cases showed brain metastases 1½ years later. In the other two fatal cases there was an increased intraocular pressure and an acute red eye as a presenting sign. In these cases tumour recurred in the eye.

e months after the diagnosis and both patients died from the disease within months of the tumour diagnosis

All cases with unilateral tumour were enucleated at the time of diagnosis. In bilateral cases enucleation usually had been performed in the eye harbouring largest tumour and the other eye was treated by local irradiation therapy. In 19 cases the second eye was enucleated either because the tumour primarily considered to be too large for radiotherapy or because changes secondary to therapy made it impossible to inspect the retina.

The time of observation varied between six and 19 years after diagnosis.

#### histological observations

Table III gives the results of the different histological variables. It can be seen that almost every tumour showed necroses and that calcifications were frequent. It may also be noted that 29% of the eyes displayed tumour in the choroid and 6% showed tumour at or beyond the lamina cribrosa. In most cases the tumour seemed to fill more than half of the sectioned area. The macroscopic descriptions however did not give any exact measure of the tumour size.

Table III  
Histological findings in the 75 retinoblastoma eyes

Tumour necrosis	97 %
Tumour calcifications	72 %
Perivascular collars	83 %
Endophytic growth	9 %
Exophytic growth	41 %
Multifocal growth	27 %
Diffuse retinal growth	4 %
Fleurettes	0 %
Rosettes	50 %
No rosettes	50 %
Mitoses	76 %
Choroidal invasion	29 %
Optic nerve invasion	11 %
Subarachnoidal invasion	1 %
Extra bulbar tumour extension	3 %
Tumour cells in anterior chamber	11 %
Retinal detachment	63 %
Inflammatory reaction in tumour	69 %
Inflammatory reaction in uvea	13 %

Only from three of the four fatal cases were eyes available for examination (3 eyes). These showed no rosettes and mitoses were prominent. Two showed tumour cells in the choroid. The optic nerve could be inspected in two cases and none showed tumour cells.

## DISCUSSION

Retinoblastoma has previously been studied by several Scandinavian authors (Horven 1926, Mork 1961, Jensen 1965, Nielsen & Goldschmidt 1967, Tarkkanen & Tuovinen 1971, Hörven 1974, Jerndal et al 1973). The frequency of the disease varies. In Finland between 1912 and 1919 the frequency was 1:82 000 live births (Tarkkanen & Tuovinen 1971). In a small Norwegian material from 1969 to 1971 comprising 16 cases a frequency of 1 per 1791 was reported (Horven 1974). However the figure 1 per 18 000 in the present material is similar to that in Denmark (Jensen 1965) and also to that in other West European countries (cf Bishop & Madson 1975).

The sex and age incidence do not significantly differ from those of other countries. The site location of the tumour is similar to that reported from other Scandinavian countries.

A familial history was rare in our material although there is some uncertainty since this investigation was limited to a perusal of the clinical records. However it may be noted that a familial history was rare in earlier Swedish reports (Jerreb et al 1967, Jerndal et al 1973). It should however be expected that a large percentage of the bilateral cases were hereditary (Sjoberg & Kimmijser et al 1966). It is noteworthy that among the six cases with a familial history of retinoblastoma there were five boys with bilateral tumours. The sixth case was a girl with a right sided tumour.

The mortality was very low and only one of the four fatal cases was bilateral. This contrasts with the higher mortality reported previously from other Scandinavian countries where the deaths were 30% in Finland, 31% in Denmark and 21% in Norway. It is difficult to explain the considerable difference. One possibility is that the therapy of bilateral retinoblastoma has improved with the introduction of the local irradiation therapy (Sjoberg 1952, Rosengren & Tengroth 1963). Neither the low mortality in the bilateral cases nor the duration of symptoms before diagnosis or the primary treatment differ significantly from those of other countries.

The histological findings did not significantly differ from other reports. The choroidal invasion in retinoblastoma has been claimed to consist

sen the prognosis of the disease (Carbajal 1958 1959 Merriam 1950) It  
however of interest to note that 29% of our cases showed choroidal tumour  
invasion. In our material this variable did not seem to influence significantly  
prognosis. This agrees with the conclusions of Redler & Ellsworth (1973).  
presence of the tumour cells in the anterior chamber or infiltration of the  
tumour into the optic nerve has not proved to be of prognostic importance in  
our material. Although tumour calcifications were very frequent in our mate-  
rial (100%) in no case was a radiograph taken.

The present study has shown that there is no significant difference in  
clinical or pathological manifestations of the retinoblastoma diagnosed in  
Sweden compared with those in other Scandinavian countries. The mortality  
never was lower probably as a result of improved radiation therapy. Al-  
though the rate of inheritance is low in the present material the findings  
never are in accordance with the suggestion that most of the cases with  
retinoblastoma are sporadic (Duke Elder 1967).

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pearance function and electroretinogram ■ follow up period of at least years to rule out delayed onset in the unaffected eye and exclusion of a inflammatory cause in the affected eye (especially syphilis or viral disease) have suggested further strictures such as the presence of a normal EOG fellow eye (Henkes 1966) Refinements in the recording and evaluation of electroretinograms developed since these criteria were suggested (for example evaluation of implicit times (Berson et al 1968)) have allowed earlier diagnosis in eyes which might previously have been considered normal

The case herein described has the clinical appearance of unilateral retinitis pigmentosa the serologic criteria for luetic retinopathy but the electrophysiologic findings of bilateral retinitis pigmentosa presenting in a monogenic manner

## Case Report

I M C a 51 year old Negro female was first seen in August 1964 with a "poor vision OD for an unknown length of time which had recently become much worse" There was no personal history of trauma infection diabetes or hypertension and no family history of visual problems Uncorrected visual acuity 20/20 Best corrected acuity OD (-0.50 combined with a +1.50 axis 100) was Pupils were felt to be normally reactive Abnormal findings were limited to the OD of the right eye and consisted of pigment clumping with spicules both in the periphery and posterior pole markedly attenuated vessels and a pale disc The differential diagnosis was luetic retinopathy vs unilateral retinitis pigmentosa A retinal consultant saw the patient and diagnosed luetic retinopathy OD

An FTA ABS was drawn and was positive (VDRL was non reactive) The patient received standard therapy consisting of 3 million units Benzathine Penicillin intramuscularly given at weekly intervals for a total of three doses (9 000 000 units) There was no evidence of a Herxheimer reaction Follow up ophthalmologic examination 6 months later was unchanged

The patient returned in January 1966 complaining of further decrease in vision Best corrected VA at this time was 20/80 +1 OD 20/40 -2 OS Fundus findings were essentially unchanged although some vascular sheathing OD was noted by the consultant The patient was presented to the same retinal consultant who stated she definitely had luetic retinopathy OD

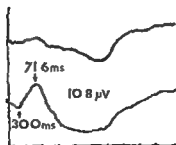
In December 1966 acuity had dropped to hand movements OD remaining at -2 OS At this time a right exotropia and amaurotic pupil OD were noted Fundus findings were unchanged and a different retinal consultant concurred in the diagnosis of luetic retinopathy OD normal fundus OS

In August 1967 acuity OD had dropped to light perception with some projection Acuity remained 20/20 -2 OS with no funduscopic abnormalities noted An ERG was obtained which revealed no recordable responses OD and normal amplitude photopic and scotopic responses OS (latencies were not determined) EOG ratios were 1.0 and 1.86 OS (normal greater than 1.50) Fundus photos were obtained OD and OS but the patient refused fundus photography OS



*Fig 1*

fundus photo OD showing marked pigmentary retinopathy pale disc and attenuated vessels with sheathing



*Fig 2*

Lower lid skin electrode ERG OU (OD above OS below) 198 responses averaged  
See Discussion for normal voltage and implicit time values



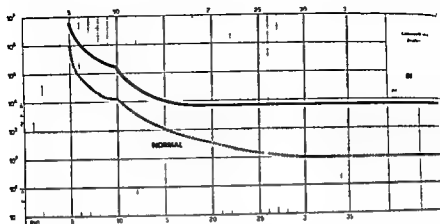


Fig 3

Dark adaptometry OS showing elevated thresholds Normal adaptation curve patient's age included for reference

We were able to examine the patient in April 1978. Best corrected vision OS was 20/20. Fundus examination was unchanged OU. The vitreous remained clear in both eyes. Goldmann visual fields showed only a temporal island OD and a superior constriction to several isopters OS. Standard ERG testing (Cambridge ERG using Burian Allen corneal electrodes) revealed non recordable responses OD and normal voltages OS. Scotopic amplitudes OS were reduced by 50 to 75% in comparison with the ERG obtained under identical testing conditions (Cambridge protocol) in August 1977. Computer averaged skin electrode ERG testing OS revealed prolongation of implicit times (Fig 2). EOG ratios were 1.00 OD 1.55 OS. Goldmann Weekers dark adaptometry OS revealed final thresholds to be elevated nearly 10 units (Fig 3). Fundus photography OS was again refused by the patient. The diagnosis was felt to be either markedly asymmetrical retinitis pigmentosa with an inconclusive positive serology or a mild form of tapetoretinal degeneration (RP) with superimposed unilateral luetic retinopathy.

## Discussion

Syphilis may produce a retinopathy whose appearance is clinically distinguishable from retinitis pigmentosa. Although acquired luetic chorioretinopathy is generally a binocular disease, the clinical presentation may be unilateral. Most commonly the disease is associated with vitreous haze and retinal vasculitis particularly affecting the arterial side of the circulation. Presence of iritis or focal choroiditis is common. Progression does not occur after adequate anti-syphilitic therapy and final visual acuity is usually fairly good (Falls 1966). While a pseudo retinitis pigmentosa appears to be present in the fundus, discrete chorioretinal lesions are usually not apparent amid the pigmentary clumps.

he diagnosis of luetic retinopathy requires serologic studies often including FTA ABS As Smith (1969) has emphasized the VDRL and even the TPI may be negative in persons with ocular syphilis It is quite likely that some earlier case reports of unilateral retinitis pigmentosa were actually cases of negative (by the less specific tests then available) luetic retinopathy Weiss & Nicholl (1968) reported two cases of unilateral retinitis pigmentosa in patients with negative FTA ABS and other serologic tests but these authors based their diagnosis on a compatible fundus appearance in one eye normal funduscopy acuity and visual fields in the other No electrophysiologic studies were performed nor were other possible etiologies (eg trauma vascular insufficiency) ruled out

While syphilis the great imitator may clinically mimic nearly any other disease the presence of a positive serology does not necessarily mean that the lesion in question is luetic in origin Serologic evidence of prior syphilitic infection should be considered a necessary although not a sufficient condition for the attribution of a luetic etiology to the pathology under consideration Complete abolition of any recordable electroretinogram would be unusual although not unknown (Smith 1969) in luetic retinopathy as would continued progression of disease after adequate treatment In luetic retinopathy as well as in other forms of inflammatory retinopathy ERG a and b wave latencies could remain normal Prolongation of implicit times appears to be a feature of inherited tapetoretinal degenerations

Characteristic of the electroretinogram in tapetoretinal degenerations (retinitis pigmentosa) are changes in the implicit times of the evoked potentials as well as in their amplitudes (Berson et al 1968) While amplitude reduction is proportional to the amount of non functional retina in inflammatory vascular traumatic and toxic affections the time course of the residual ERG response is unremarkable The tapetoretinal degenerations on the other hand show prolongation of the temporal course of electrophysiologic responses to light Amplitude reductions vary with most forms of retinitis pigmentosa showing early and profound abnormalities while some incomplete forms show relatively mild reductions in amplitude Pruett & Schwarz (1978) have recently described three cases of occult retinitis pigmentosa where visual symptomatology did not become manifest until the seventh decade of life

We have previously shown (Skalka in press) that photopic computer averaged skin electrode ERG responses with the Grass photostimulator lamp at S16 intensity and placed four feet from the patient's eye show a wave implicit times of  $23.8 \pm 3.68$  ( $\pm 1$  SD) msec and b wave implicit times of  $49.92 \pm 4.17$  ( $\pm 1$  SD) msec As seen in Fig 1 our patient's a wave implicit time OS of 30.0 msec and b wave implicit time of 71 msec show a wave prolongation of nearly

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# INTRAMUSCULAR IRON THERAPY AND TAPETORETINAL DEGENERATION

A case report

BY

KJELL SYVERSEN

A 63 years old male with pernicious anemia had been treated for 20 years with weekly injections of iron dextran and cyanocobalamin. Ophthalmological examination revealed the ophthalmoscopic picture of a tapetoretinal degeneration. reduced visual acuity and narrow visual fields. ERG and dark adaptation test were normal. Hematological examinations including liver and bone marrow biopsies gave no support for the existence of systemic siderosis. It is proposed that the retinal degeneration is due to the extensive parenteral iron treatment with a total dose of approximately 100 grams of iron. This theory is supported by a previous experimental report.

**Key words:** Retinitis pigmentosa - tapetoretinal degeneration - pernicious anemia - parenteral iron treatment - systemic siderosis

In an experimental work Gibis et al (1951) described defective vision and retinitis pigmentosa like retinal disease in dogs 4 to 7 years after repeated injections of iron oxide or multiple blood transfusions. The dogs showed signs of hepatic cirrhosis or findings similar to hemochromatosis. All the who had been kept alive for 7 years after the iron treatment had severe degenerations in their retinas but only small amounts of iron deposits.

In the same work Gibis et al reported the findings of siderotic deposits in the retina, the pigment epithelium, choriocapillaris and the ciliary bodies.

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ent who had been treated with multiple blood transfusions I have not been able to find any report of eye damage in man caused by parenteral iron therapy or hemochromatosis

## Case Report

Male farmer born 1914 from unrelated parents and with no family history of retinitis pigmentosa or other eye diseases. In 1941 gastric resection was performed because of a peptic ulcer. A pernicious anemia was diagnosed in 1957 and since then has been treated with cyanocobalamin preparations in adequate doses. Additionally received weekly intramuscular injections of iron dextran (R Imferon) each injection containing 50 mg Fe/ml. As this treatment was given uninterruptedly for 20 years total iron dose has been approximately 100 grams.

He had no visual impairment until 1973 when he first complained of reduced vision and poor night vision. At the time of his first visit to our department in May 1974 general health was good. Blood pressure 150/90. Our standard hematological tests including hemoglobin, serum iron and serum Vitamin B<sub>12</sub> were normal. There were no brown dust like materials on the back surfaces of both corneas and a moderate thickening of the trabecular meshworks. Both lenses had pseudoexfoliation of the anterior capsules but no cataract or discolouration. Ophthalmoscopy and fundus photography was to some extent difficult due to a diffuse obstruction to the light transmission in the vitreous bodies. No distinct opacities could be observed. Nevertheless it was possible to observe the optic discs which were both of normal colour with physiologic cupping. The retinal arteries were slightly narrowed artery/vein relation = 1/2. In the equatorial regions the retinal epithelium showed marked atrophy in multiple well demarcated patches and dark brown pigment deposits with bone spiculation.

Visual acuity was R Esph -1 cyl 90 -1 1/2 L Esph -0 1/2 cyl 90 -1 5/8 5. Visual field examination with Goldmann perimeter and Hamblin perimeter revealed narrowing of visual fields to 10-15° in both eyes. Colour vision was normal tested with Ishihara's pseudoisochromatic charts and Farnsworth 15 hue test.

ERG and dark adaptation tests were not available in the department and the disease was primarily considered to be an idiopathic retinitis pigmentosa. Little attention was paid to the hematological aspects as the anemia was kept under control and treated by his general practitioner.

During the years 1974-1976 there were no visible changes in the retinal pictures except a gradual loss of vision and peripheral visual fields. In Dec 1976 the corrected vision was R E 5/10 L E 5/10. Up to that time the treatment with cyanocobalamin and intramuscular iron dextran had been continued. In January 1977 a blood examination revealed a slight elevation of the ESR (24 mm/h). Hemoglobin, hematocrit, serum iron, bilirubin, alkaline phosphatase, ASAT, ALAT and serum Vitamin B<sub>12</sub> were normal.

The iron-binding capacity was slightly decreased (47 mg/l). The treatment with cyanocobalamin was kept unchanged but iron dextran was discontinued from Jan 1977. Examination in the dept of internal medicine in March 1977 confirmed the diagnosis of pernicious anemia; the hematological values showed no changes from those mentioned above. Needle biopsy from the liver showed fatty degeneration and

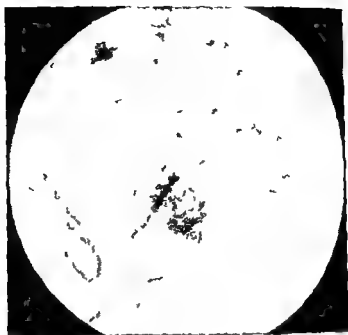


Fig 1

Fundus photograph from right fundus

a special staining for iron revealed some degree of iron pigment deposition siderosis. Liver scintigraphy was normal. The conclusion from the medical history was that the patient had a well balanced pernicious anemia and no sign of pathological iron storing.

Since March 1977 there has been no progression of the visual loss and in the last five examinations up to Dec 1978 an improvement of the visual acuity and the visual fields have been noted. At the last examination, the V.A. was R.E. 5/6. A reduction in the pigmentations or the atrophic changes in the retina has not been observed. ERG and dark adaptation test in June 1978 (Dept of Ophthalmology Bergen University) were normal. A drop in the hemoglobin in March has responded well to per oral iron medication.

## Discussion

The coexistence of pernicious anemia and tapetoretinal degeneration as two independent diseases cannot be excluded but it is not inconceivable that there might exist a connection between them though I have not been able to find a report of any such coexistence or retinotoxic effect of cyanocobalamin. Parenteral iron therapy is usually recommended for a limited period of

in order to fill the iron depots of the body. The effect of more prolonged treatment is not fully known.

In our case the patient received a total dose of approximately 100 grams parenterally. The body has a very limited capacity to excrete iron and thus accumulation must have been considerable. There is however no sign of pathologic storing of iron in the liver or bone marrow.

The tapetoretinal degeneration in spite of no pathologic iron storing corresponds well to the clinical picture in dogs demonstrated by Cibis et al (1957).

This indicates a retinotoxic effect of prolonged treatment with parenteral iron. Lack of progression and even slight improvement during the time after discontinuation of the iron therapy may support a causative relationship. ERG and dark adaptation test were made at a stage of clinical improvement but the final findings speak against retinitis pigmentosa. Our patient received an extremely high total dose of parenteral iron and similar cases will probably be found far between.

The possibility of eye complications should be born in mind when giving prolonged treatment with iron injections or multiple blood transfusions.

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## UNUSUAL OCULAR MANIFESTATION IN TEMPORAL ARTERITIS

A case report

BY

NIELS VESTI NIELSEN and JENS SINOBERG ERIKSEN

Transitory oedema of the left orbit and maxillary sinus was revealed radiographically in an active phase of temporal arteritis. This is possibly caused by inflammatory changes of the soft tissues in these regions. Early signs of compromised retinal flow and anterior ischemic optic neuropathy were demonstrated with fluorescein angiography. This finding promoted early immediate therapy.

**Key words:** temporal arteritis radiographically orbital oedema - fluorescein angiography - intraocular pressure

Ocular complications of temporal arteritis are frequent - 32-100 per cent (Parsons Smith 1959 Whitefield et al 1963). Sudden loss of vision without means rare (20-72 per cent) owing to ischemic neuropathy of the optic nerve or occlusion of the central retinal artery (Heptinstall et al 1954 Wamholt Hollenhorst 1958 Havreh 1975).

Less frequent and more atypical manifestations have been reported: extraocular muscle palsy, visual hallucinations, hypotonia bulbi and infarctions of the anterior eye segment - (Cullen & Coleiro 1946 Verdich & Nielsen 1973).

No previous reports have described the appearance of radiographically detectable orbital and maxillary oedema in temporal arteritis.

## Case Report

an aged 50 years was admitted to hospital on March 31 1978 with a diagnosis of  
infectious fever ESR 105 mm/h and haematuria

### Present History

The patient had fever for 10 days prior to his admission  
Otitis was suspected and he was treated with antibiotics for seven days with no  
response  
During the last six days before admission the patient had complained of a smarting  
burning pain of the tongue scalp pain frontal headache and intermittent left  
visual disturbances in the form of rainbow vision

### Past History

History of eye diseases

The patient had been admitted in 1957 with haematuria for which no underlying  
cause was detectable In 1962 admitted with spondylosis of the cervical spine and in  
1963 with an injured back In 1965 the patient had a slipped lumbar disc  
He had been well since then

### Physical findings on Admission

General condition was unaffected Blood pressure 140/100 pulse rate 84 temp 38.5°C  
On the skull there was tenderness of left superficial temporal artery Both arteries  
were somewhat thickened but with good pulsation  
Cystoscopy of the urinary bladder disclosed one large and two small diverticula  
in which blood exuded. Puncture of the left maxillary sinus disclosed absence of  
infection  
Physical examination revealed no other pathological phenomena

### Ophthalmologic consultation April 1 1978

His corrected visual acuity was almost 6/6 in both eyes  
Visual field (campimeter) 3/1000 mm no scotoma nor defects  
There was no protusion of the eyeballs The pupils reacted normally to light  
Slit lamp examination disclosed dilated iris vessels of left eye Intraocular tension  
was 15 mm Hg in both eyes with Goldmanns applanation tonometry  
On ophthalmoscopy both papillae were normal Both retinae showed mild arterio  
sclerosis and incipient degenerative changes in the posterior pole  
The ophthalmologist recommended biopsy of the temporal artery x ray examination  
of sinuses and treatment with prednisone owing to suspicion of temporal arteritis

### Laboratory Tests on Admission

Haemoglobin 7.0 mmol/l Leucocytes 15.5-10<sup>9</sup>/l ESR 45 mm/h Asp aminotransferase 50 U/l  
Creatinine 101  $\mu$ mol/l Urine microscopy numerous erythrocytes Culture from  
stool streptococcus faecalis Serum protein quantitative immunologic Albumin 21 g/l



(34-53 g/l) Transferrin 10 g/l (20-27 g/l) Haptoglobin 53 g/l (0.2-2.1 g/l)  
 g/l (7.8-16.0 g/l) IgA 7.6 g/l (0.8-4.4 g/l) IgM 0.7 g/l (0.5-2.6 g/l) Orosomucoid  
 (0.5-1.1 g/l) Complement C - 3.20 g/l (0.8-1.5 g/l)

We found negative titres on examination of AST and ASH rheumatic factor,  
 no auto antibodies. Cultures from blood were negative.



Fig 1

X-ray of the orbits and sinuses. A blurring of the left orbit and maxillary sinus  
 revealed during an untreated phase of ocular temporal arteritis.

*ay of sinuses* no sinusitis but slight diffuse blurring of the left orbit and maxillary sinuses (figure 1) Control radiography of the sinuses and orbits three days later showed blurring to have subsided

*ay of thorax* mild pulmonary emphysema Sequels of a fracture of left eighth rib

### *G normal*

After two days in bed there were no complaints of headache or visual disturbances Prednisone treatment was postponed owing to the suspicion of an infectious disease and uncertainty regarding the diagnosis of temporal arteritis Results of biopsy of the superficial artery performed on April 2 1978 was not available for 10 days On April 10 the patient was referred to the Eye Department for correction of existing glasses The day before the patient had noticed intermittently blurring vision on standing posture There was no concurrent headache Temp was 37.9°C and ESR 1 mm

### *ocular State*

Visual acuity in the left eye was reduced to 6/15 best corrected No tenderness to the temporal region was noticed but marked dilation of the blood vessels of conjunctiva and sclera The pupils reacted normally to light Slit lamp examination disclosed clearly dilated iris vessels of the left eye without aqueous flare Intraocular tension was 16 mm Hg on the right and 11 mm Hg on the left eye using Goldmanns applanation tonometry Ophthalmoscopy of the right eye was unchanged but in the left eye the peripapillary region was characterized by slight blurring and the superior macular arterioles were closed by small exudates The venules were slightly dilated and darkened Fluorescein angiography of the left eye revealed delayed filling of the retinal vessels and hypofluorescence of the optic disc In addition points of leakage in the centre of retina were seen (Fig. 2)

### *treatment*

Hydrocortisone (dexamethasone) 6 mg injected intravenously Prednisone tablets 20 mg four times daily Theophyllamine 700 mg injected intravenously Intravenous theophyllamine drop 1 g/1000 ml of glucose over 24 hours Diamox capsules (acetazolamide) 500 mg once daily Histological examination was available the same day but after treatment had been instituted. It showed the left superficial temporal artery to have undergone changes compatible with a diagnosis of temporal arteritis Next day subjective improvement of general condition and vision had already occurred Temp 37°C Left eye vision was improved to 6/15 The vessels of the conjunctiva and iris still were dilated and intraocular tension was 12 mm Hg in the right and 11 mm Hg in the left eye



*Fig 2 a b*

Fluorescein angiography of the left eye. The choroidal and retinal circulation was slightly impaired (visual acuity 6/15) during untreated ocular temporal arteritis (Fig 2a). Following treatment for one day circulation had been improved (visual acuity 6/7.5) - Fig 2b.

Fluorescein angiography was repeated. It showed an improved filling of the retinal vessels (Fig 2).

The patient was discharged on April 20, feeling well with no complaints and a visual acuity ESE 5 mm/h.

#### **Course**

Monthly controls and two repeated fluorescein angiography examinations of the eye over the next four months showed no relapse.

#### **Discussion**

A slight radiographically oedematous blurring of the left orbit and paranasal sinus was revealed in this patient. The oedema may be assumed to consist partly of the exudative changes due to arteritis in the orbital and paranasal arteries.

The orbital oedema remarkably did not cause protusion of the eyeball. Both maxillary and orbital oedema were transient. Thus the radiographical ring had disappeared after three days of confinement to bed without any local treatment. Moreover the general health was simultaneously improved. The clinical findings probably indicate that the maxillary and orbital oedema in this case was caused by temporal arteritis. No previous reports are available of these radiographic changes in temporal arteritis. It is however a well known fact that clinical signs of orbital involvement in arteritis temporalis can be seen (Bettelheim 1968).

In our case confinement to bed had a favourable influence on the signs and symptoms of arteritis (visual disturbances and somatic complaints). These disappeared when the patient changed from a recumbent to an erect posture. Ellenhorst (1967) had also observed a correlation between fluctuations of eye symptoms and postural alterations in temporal arteritis. This phenomenon is probably due to a critical reduction of a marginal flow to the eye in the erect posture.

As the left eye was involved during an aggravation of temporal arteritis a reduction of the intraocular pressure of the affected eye was disclosed. Hørvén (1968) accordingly has noted a reduction of intraocular pressure as a sign of ocular involvement in temporal arteritis in 22 patients. Furthermore the occurrence of hypotonia bulbi in temporal arteritis has been reported by Bettelheim 1968 Daicker & Keller 1961 Goder 1968 Haimbok 1961 and Erdich & Nielsen 1965.

In our case an early fluorescein angiographic diagnosis of impaired retinal circulation and an ischaemic optic neuropathy promoted immediate treatment.

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## **STIMULUS ALTERNATION AND FAST RETINAL POTENTIALS PHOTOPIC AND SCOTOPIC CONTRIBUTIONS\***

BY

MATTHIAS KORTH and VIKTOR REIMAN

Human retinal oscillatory potentials in response to an alternating checker board pattern stimulus were studied in two subjects over an intensity range of 7.5 log units. Under scotopic conditions two wavelets ( $S_1$  and  $S_2$ ) could be recorded. At an intensity of 1.9 photopic log Td four high frequency oscillations ( $O_1$ – $O_4$ ) were noticed and a discontinuity was observed in the corresponding luminance curve of the b wave together with a sudden decrease in the magnitude of the standard deviation of the amplitude measures. The oscillations were noticed only on the ascending slope of the b wave. With increasing stimulus intensity their latency decreased at a slower rate than that of the b wave and their number decreased. Each wavelet had an amplitude maximum at a certain stimulus intensity level. It was suggested that  $O_1$ – $O_4$  were generated by the activation of the photopic system and that  $S_1$  and  $S_2$  were of scotopic origin.

**Key words:** averaged ERG – electroretinography – ERG wavelets – oscillatory potentials – pattern reversal – photopic ERG – rod cone break – scotopic ERG – stimulus alternation

Since the first description of the retinal oscillatory potentials by Cobb & Morton (1954) many experiments have been concerned with their sites of origin and mechanism of generation. Further much research has been concerned with the question whether these oscillatory waves were triggered by the activity of rods or cones. Results from several studies have indicated that the oscillations

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oscillations were probably of photopic origin. In several experiments to control the state of adaption oscillatory potentials in response to flashes of light were obtained (De Molfetta et al 1968, Jacobson et al 1970, Algvere & Westbeck 1972) suggesting their photopic nature. The experiments utilizing coloured light stimuli (Alfieri & Sole 1968) and selective adaptation (Heck & Rendahl 1957, Rendahl 1958, 1964) and the close relation of the oscillations with the photopic system. Further the fact that in colour blind individuals no oscillations can be recorded (Heck & Rendahl 1957, Goodman & Bornschein 1957) is also in line with these conclusions.

In his clinical studies Algvere (1963a,b) noted that in patients with disorders of the central retina the oscillations were more likely to be missing than in patients with diseases of the peripheral retina. This finding suggests that the oscillations are initiated by the cone system.

Results of other experiments however seem to indicate that the rod system also contributes to the generation of oscillatory waves. Auerbach after examining the wavelets during dark adaption ascribed some of them to photopic and others to scotopic activity. Based on light and dark adaptation studies Algvere & Wachtmeister (1972) and Wachtmeister (1972) concluded that the contribution of the rods to the generation of the oscillations could not be ruled out since they were most pronounced under scotopic states of adaptation.

The somewhat contradictory conclusions from the experiments reviewed above may be due to the utilization of different procedures. Also the knowledge of the state of the subject's adaptation seems to be very important in order to judge the character of the oscillatory potentials.

In the present experiment the method of stimulus alternation (Jägle 1966) was used. This procedure allows a good control of the eye's state since test and adaptation light are identical and the luminous flux entering the pupil is kept constant throughout a recording session. The stray light caused by conventional flashes of light is avoided thus leaving the on and off response restricted to the directly stimulated retinal area. Further a large number of sweeps were averaged in the present study in order to obtain reliable responses at stimulus intensities lower than the intensity levels that have typically been examined.

## Methods and Material

The stimulus consisted of a circular patch of a spatially alternating checkerboard pattern presented in Maxwellian view. The diameter of the visual field was  $46.5^\circ$  the size of one checkerboard element was  $2.44'$  and a fixation mark was in the center of the display.

intensity of the white light of a 75 Watt Xenon high pressure arc lamp (Osram) controlled by means of neutral density filters (Schott). The maximum retinal illuminance produced by the bright checkerboard elements as measured according to a method described by Westheimer (1966) using a digital photometer (Tetronix J16) photopic log Td for the lowest intensity level tested the corresponding value 63 scotopic Td.

The method of stimulus alternation has been described elsewhere (Armington 1968). In the present experiment an optical scanner (General Scanning G100PD) moved the checkerboard back and forth in a square wave fashion. Each movement was completed in 700  $\mu$ sec and the alternation rate was two per second. With each displacement of the stimulus pattern the total amount of light flux entering the pupil changed by 15%.

An electroretinogram (ERG) was recorded from the right eye with a silver electrode in contact with the eye by a scleral contact lens. The right ear was used as reference; the left ear was grounded. After amplification (Tektronix 122 and Tektronix 509 frequency response 0.2 Hz–10 kHz) the potentials were stored on an analog tape recorder (Sanborn model Sabre VI) together with the trigger signal of the square wave generator (Hite model 5300 R) which drove the amplifier (General Scanning CCY 101) of the optical scanner. Later the tape recorder was played back twice into a digital computer (PDP 11/40) and response averaging was performed with two different sweeps. 512 sweeps were accumulated for each intensity setting and the responses to the back and forth movement were added together.

All experiments were carried out in an electrically shielded and light proof experimental chamber. After a dark adaptation period of 30 min stimuli were presented in increasing intensity in steps of 0.5 log units. Stable head position was insured by means of a dental impression bite board. Identical sessions applying the same sequence and range of intensities were repeated six times.

Seventeen healthy experienced male subjects (M K and V R) were used. The slow and fast ERG components were evaluated with sweep lengths of 400 ms and 100 ms respectively. In both cases the sweep comprised 512 points. In order to isolate the individual components the short sweep records were digitally filtered so as to eliminate the low frequency components. The smoothed function thus obtained was subtracted from the unfiltered record thereby leaving only the fast potentials.

To compute the smoothed function a three point Hanning filter of the following form was used:

$$S(0) = 1/2 Y(0) + 1/2 Y(1)$$

$$S(i) = Y(i-1)/4 + Y(i)/2 + Y(i+1)/4$$

$$S(n) = 1/2 Y(n-1) + 1/2 Y(n)$$

$S(0)$  represents the first data point

$S(n)$  represents the last data point

$S(i)$  represents the intermediate points that have been smoothed

$Y(i)$  represents the value of the data point being smoothed

For the 10 ms sweeps the above algorithm was repeated 1000 times resulting in an averaging of frequencies above 100 Hz by a factor of at least 100 (Fig. 1).



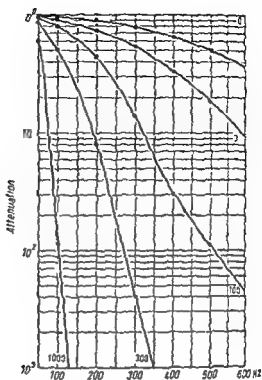


Fig 1

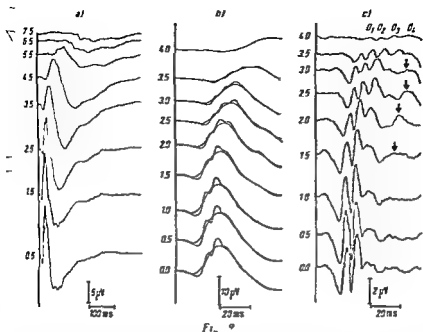
The degree of attenuation of the three point Hanning filter as a function of frequency. The parameter is the number of repetitions of the filter algorithm. Sweep length = 100 ms. The number of data points was 512.

The amplitude of the b wave was measured from the lowest point of the a wave to the peak of the b wave. The amplitude of the high frequency components was determined as the magnitude of the peak of a wavelet relative to the preceding trough of a high pass filtered record.

## Results

Fig 2 presents three sets of ERC's collected from subject M. K. with three different stimulus intensities and with sweep lengths of 420 ms (a) and 10 ms (b and c). In b the smoothed b wave was plotted together with the original record. Fig 2c shows the difference between the filtered and the unfiltered short sweep ERC's.

At the very low luminance levels (Fig 2a) the response consisted mainly of a slow negative going late receptor potential. A b wave appeared at somewhat higher stimulus intensities. Both the late negative potential and the b wave



filtered and filtered ERGs with two different time resolutions under different levels of adaptation. The numbers along the ordinates indicate the values of the neutral density filters. a) Unfiltered long sweep ERGs covering the whole intensity range tested. Photopic unfiltered and superimposed low pass filtered short sweep records. c) High pass filtered records obtained from b) by subtracting the low pass filtered from the unfiltered trace. The arrows indicate a potential which was probably of scotopic origin. All traces are averages of six trials with a total of 30  $\pm$  2 sweeps. The reversal of the stimulus pattern took place at the vertical line shown at the beginning of each trace.

re superimposed by a few wavelets which became less distinctive as the stimulus level was raised further. Multiple waves resembling the typical oscillatory potentials began to appear again at the intensity given by the 4.0 neutral density filter (Fig. 2b,c).

In Fig. 3 the amplitude of the ERG b wave was plotted as a function of stimulus intensity. The resulting graph shows two important properties: (1) there was a discontinuity in the curve with a plateau between the filter settings 0 and 3.5; (2) The magnitude of the standard deviation of the b wave amplitude showing a steady increase with stimulus intensity abruptly diminished at filter 4.0. However, with further increase in the stimulus level it increased monotonically again. The discontinuity in the amplitude values and in the standard deviation suggested the presence of two different processes. Thus, in

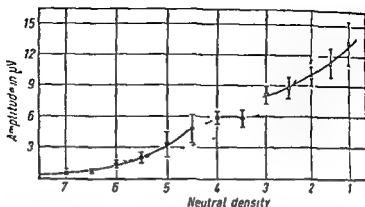


Fig 3

Luminance curve of the EERG b wave obtained from subject M. The data are averages of six trials. The lower left and upper right smooth curves represent exponential fits to the filled and open circles respectively. The two half filled circles are excluded from either curve fitting. The dashed curve is an exponential fit to all data points shown. The vertical bars represent the magnitude of the standard deviation.

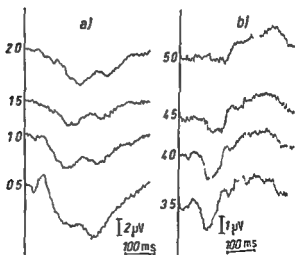


Fig 4

Averaged EERGs obtained with red light (a) and under foveal stimulation (b). Numbers indicate the values of the neutral density filters. At filter setting 1.0 (a) and 4.0 (b) the retinal illuminance was 2.0 and 1.9 photopic log Td respectively. The curves represent averages of 1024 (a) and 1048 (b) sweeps.

3 two separate exponential functions were computed and fitted to the data according to the method of least squares. The filled circles of Fig. 3 were for fitting a scotopic and the open circles were used for fitting a photopic. The half filled circles representing the plateau were not taken into account for any computation since they could not be ascribed satisfactorily to a process. The dashed curve in Fig. 3 was included to show that a single exponential function could not accurately describe all the data points.

In order to determine the absolute threshold of the photopic b wave two additional experiments were performed using the right dark adapted eye of subject M. K. In the first one a red stimulus light of 670 nm (grating monochromator Schoffel model GM 250) with otherwise unchanged stimulus specifications was used. In the second experiment a circular stimulus of  $4^\circ$  in diameter containing a colourless checkerboard pattern with an element size of  $2\phi$  centered on the fovea. The original light beam without the monochromator used in Fig. 4 shows that a small b wave preceding the negative after potential could be distinguished for the red stimulus (a) at filter 10 and for the normal ERG (b) at filter 40. The corresponding retinal illuminances were 2.0 and 1.9 photopic log Td respectively. At those threshold intensities the b waves obtained in the two experiments had identical peak latencies which in both were equal to the peak latency of the b wave obtained in the main experiment at filter 40 (Fig. 2).

The luminance curve obtained from subject V. R. had an almost identical shape and the discontinuity of the curve occurred at the same stimulus intensity. The amplitudes of the different fast components of the ERG shown in Fig. 2 exhibited a somewhat more complicated behaviour as intensity was raised. The upper half of Fig. 5 shows that with increasing stimulus intensity the oscillations increased in amplitude up to a maximum which occurred for each wavelet at a different level of adaptation. The amplitude of the smoothed b wave and the steepness of a straight line between the lowest point of the potential wave shortly after stimulus onset and the highest peak of the b wave are shown for comparison in the upper half of Fig. 5. The course of the amplitude of the first wavelets resembled that of the amplitude and the steepness of the ascending slope of the filtered b wave. From the agreement between amplitude and steepness of the filtered b wave it follows that the time to peak (as measured from the beginning of its onset) declined while its amplitude increased (i.e. the spectral composition of the b wave shifted towards higher frequencies with rising stimulus intensity).

Fig. 6 shows that the peak latencies of the wavelets decreased with increasing stimulus intensity at a slower rate than the peak latency of the smoothed b wave. Across the intensity range during which the wavelets could be observed

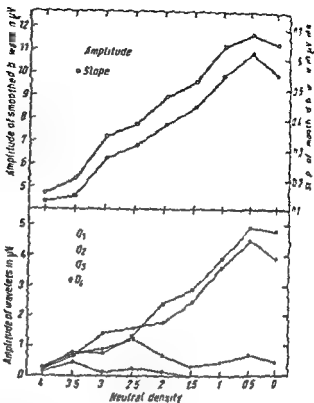


Fig. 5

Upper part: Amplitude and slope of the low pass filtered photopic ERG b wave as a function of stimulus intensity

Lower part: Concurrent luminance curves of the four oscillatory wavelets

the average time between the first and the second wavelet was 64 ms, between the second and third 81 ms, and between the third and fourth wavelet 91 ms.

The records shown in Fig. 2b indicated that the oscillations could be observed more easily when the initiation of a wavelet occurred simultaneously with the rising segment of the smoothed b wave. As soon as the peak of the smoothed b wave became shorter than that of a particular wavelet, the amplitude of the wavelet started decreasing. However, the stimulus intensity could not be reduced sufficiently so that the peak of the b wave occurred earlier than the second wavelet (Fig. 6).

Fig. 7 shows superimposed original and smoothed traces (Fig. 2a) and high pass filtered records (Fig. 1b) obtained under scotopic conditions. The smoothed records were obtained by repeating the digital filtering algorithm 200 times at a sweep length of 425 ms. At the very low intensities,

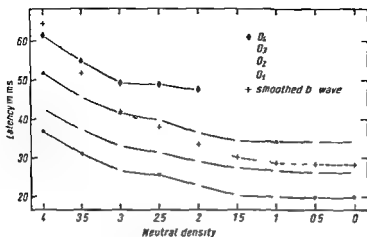


Fig 6

Peak latency curves of the four oscillatory wavelets and of the low pass filtered photic ERG b wave as a function of stimulus intensity

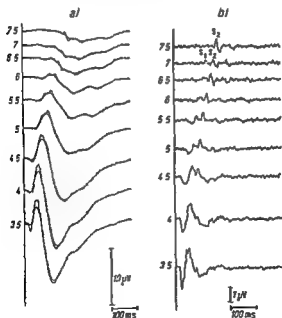


Fig 7

Unfiltered and filtered long sweep ERGs obtained under scotopic conditions. The numbers along the ordinate refer to the values of neutral density. a) Unfiltered and superimposed low pass filtered records b) High pass filtered records obtained from a) by subtracting the low pass filtered from the unfiltered traces. Regarding wavelets  $S_1$  and  $S_2$  refer to text. Averages of six trials with a total of 3072 sweeps

wavelet ( $S_1$ ) was superimposed on the early part of the late receptor potential. At filter 7.0 the b wave appeared accompanied by another wavelet  $S_2$  preceded  $S_1$ . As stimulus intensity was increased the peak latencies of the waves became shorter the time span between them (average 0.4 ms) and amplitude did not change consistently. At filter 4.0 it was impossible to make a distinction between the b wave and  $S_1$  wave  $S_2$  however could be observed on the falling slope of the b wave as stimulus intensity was increased. Under photopic conditions its amplitude increased (Fig. 2c arrows) up to intensity given by filter 2.0 and its waveform was composed of lower frequencies than the high frequency oscillatory waves.

The same number and a similar behaviour of the scotopic wavelets was found in subject V.R.

## Discussion

The results of the present experiments clearly indicate that under both photopic and scotopic conditions wavelets could be recorded. However the spectral composition and dependency on the stimulus intensity were different under the two conditions. With increasing stimulus intensity typical oscillatory potentials were observed for the first time at an intensity of 1.9 photopic trolands (density 4.0 in Fig. 2). The discontinuity in the luminance curve of the b-wave accompanied by a sudden decrease in the magnitude of the standard deviation (Fig. 3) occurring at this particular intensity could suggest that another process, probably the photopic system, might have dominated the response. This view is in agreement with the results obtained by Armington et al. (1965) who found that stimuli of 100 Td can be expected to yield photopic responses. Luminance curves that show a discontinuity separating a scotopic from a photopic branch have been obtained before using white (Goodman & Bornschein 1961) or coloured stimuli (Korth & Armington 1976).

The view that the intrusion of the photopic system might be accompanied by oscillatory potentials is supported by the records obtained with red light under foveal stimulation (Fig. 4). It can be assumed that under these conditions the responses were controlled predominantly by the activity of the cone system. In two experiments a b wave appeared at photopic intensities which were identical with the intensity at which the discontinuity in the luminance curve was observed.

The plateau formed by the two half filled data points of Fig. 3 is a clear indication of a mesopic state of adaptation where an interaction between the scotopic and the photopic system takes place. Thus it is suggested that

urrence of the oscillatory potentials ( $O_1$ – $O_4$ ) depends on the processes of the photopic system. No such high frequency oscillations were observed under scotopic conditions. However, they may occur while both systems are active at the same time.

In order to understand the mechanisms leading to the generation and suppression of the wavelets the following three observations should be emphasized. (1) The occurrence of oscillatory waves on the ascending slope of the b wave noted by Bornschein & Goodman (1957). (2) Cobb & Morton's observation (1964) of a faster decrease of the latency of the b wave than that of the wavelets, suggesting the presence of two different mechanisms as assumed also by Robson et al. (1964) and by Wachtmeister (1973). (3) From observations 1 and 2 it can be concluded that the amplitude of the oscillations having a longer latency than the peak of the b wave decreases to zero as was described by Rendahl (1958). Genest (1964), Algvere & Wachtmeister (1972) and Tsuchida et al. (1973). These three observations were supported by the data of the present experiment (Fig. 2b, c) using an alternating stimulus. The falling slope of the b wave seemed to be associated with a mechanism damping the oscillations. The onset of this damping mechanism occurred with a shorter latency as stimulus intensity was increased. The combination of these processes thus explains the different amplitude maxima of wavelets  $O_3$  and  $O_4$  (Fig. 5).

The complete absence of oscillatory potentials in cases of diabetic retinopathy (Yonemura et al. 1962; Simonsen 1965; Nakajima and Sugimachi 1963) and the observation that the oscillations are more sensitive to the presence of disease than the b wave (Algvere 1968a, b) supports the notion that the b wave is not triggered by or composed of oscillations. The fact that oscillations have never been observed without the b wave indicates that the mechanism generating the wavelets depends in some way on the processes responsible for the generation of the b wave.

The mechanism of origin of wavelets  $S_1$  and  $S_2$  probably is different from that generating the fast oscillatory potentials since they occurred under scotopic conditions and their amplitude and the time interval between the two wavelets did not depend on the stimulus intensity. Also the waveforms of  $S_1$  and  $S_2$  are composed of lower frequencies and  $S_2$  was observed on the falling slope of the b wave. Rendahl (1958, 1964) regarded the fifth wavelet as being of photopic nature, whereas Auerbach (1964) suggested that the first three wavelets were of photopic and the fourth and fifth wavelet were of scotopic origin. According to the present results, photopic oscillations and one scotopic wavelet occurred at the same adaptation level with a considerable overlap of stimulus intensities.



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# FAMILIAL CEREBRO MACULAR DEGENERATION (THE STENGEL-BATTEN MAYOU SPIELMEYER VOGT STOR DISEASE) EVALUATION OF THE PHOTORECEPTORS

BY

EGILL HANSEN

In a material comprising seven patients with familial cerebro macular degeneration a grave visual disability had developed within a few years after the onset of the disease this being at 3½ to 7 years of age. The night vision was only moderately reduced. A severe red green colour vision defect was demonstrated in three patients. This is in accord with the loss of red and green cone responses found in one patient by chromatic adaptation studies. On the other hand a remarkably good response of the blue cone system was registered. Normal pulse amplitudes were found by dynamic tonometry in four patients indicating good choroidal circulation. This supports the theory that the degeneration of the neuroepithelium is of primary type. The selectivity in loss of response functions being demonstrated here might however also be related to parallel degeneration taking place in ganglion cells.

*Key words:* juvenile amaurotic family idiocy - colour vision - dark adaptation - selective chromatic adaptation

Familial cerebro macular degeneration or juvenile amaurotic family idiocy is a neuronal ceroid lipofuscinosis with early manifestations in the eye. The disease which typically begins at the age of 5-7 years usually leads to blindness at an early age. Symptoms from the central nervous system appear and progressive dementia and paralysis occur. In most cases death follows before the age of 10 years.

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In the Scandinavian countries the disease is known under the term Spielvogt or the Spielmeier Stock disease and in England as the Batten disease. A better term is familial cerebro macular degeneration, as has the eponyms of Batten (1903) and Mayou (1904) or Spielmeier (1905) (1905) and Stock (1908) do not represent the first description of the disease. An excellent description of the disease was already given in 1826 by Christian Stengel, a general practitioner at Roros, Norway, in his report of a curious disease in four siblings in the neighbourhood of Roraas, Nissen (1904) drew attention to this early description of the disease.

The incidence of the disease is about 1/50 000 of living children born (Ray, 1969) being transmitted as an autosomal recessive trait. In the Norwegian register of the blind there are 19 known cases below the age of 15 years giving prevalence in the same population group of 2 per 100 000.

It is the purpose of the present article to report some patients examined at our department with special emphasis on the findings which might serve to characterize the affection of the photoreceptors at early stages of the disease.

## Material

The patients suffering from familial cerebro macular degeneration are affected at a young age; extensive examinations are often impossible. Besides the mental disturbances which may be present, the grave deterioration of vision by itself reduces the patient to despair. For instance, in a 5-year-old boy, because of his reduced vision, confrontation with colours which he was no longer able to see caused him to cry, and the examination had to be broken off. Certain examinations could be carried out on other patients. However, it is exceptional for young children so influenced by their disease to be able to cooperate in examinations requiring their attention over a prolonged period. Our patient I happened to be such a patient. The findings in the present report for the most part refer to this patient, but are supplemented by findings obtained with the other patients. The following patients were examined:

*Case 1 (C.C.)* a girl was nearly 7 years old at the first examination. There had been a gradual reduction during the last 1-2 years. The visual acuity was finger counting 4 and 2 in the right and left eye respectively. She had normal visual field limits but large central scotomas. There was pathological pigmentation of salt and pepper type in the macula and a Bull's eye appearance of the macula. The optic discs were slightly pale. Initially the vessels were normally calibrated. The patient had experienced difficulties with colour discrimination, especially red and dark were confused. She also confused light green and yellow as well as blue and purple. During the observation period her twilight remained quite good.

**Course** The visual acuity was gradually reduced. At the age of 19 years, she saw hand movements only in the temporal fields. The atrophic changes in the retina were more pronounced and the vessels were distinctly thin. There was a reduction in the intellectual functions. She became more apathetic and slow. At 25 years there had been two grand mal fits. The patient was also examined at the Hospital for Sick Children, London. A rectal biopsy was taken and suggested Mayhew's disease.

**Patient 2 (T.S.)** a girl had complained of eye problems since the age of 6 years. A slight reduction of visual acuity had been recorded. Visual acuity was 20/60 at the age of 14 years when she was admitted to the hospital. Good visual fields were found by perimetry though with large central scotomas. Mottled pigmentation was found in the central fundus. By an unconventional registration of dark and light slightly increased light thresholds were found during a 90 min dark adaptation. A relatively better night vision than day vision had been noticed by the patient as well as by her parents. This was the case even at an advanced stage of the disease. From the age of 17 years the patient was forgetful and infantile. Considerable mental deterioration had occurred by the age of 21 years when she had had some epileptic fits. She was dysphasic and unsteady.

**Patient 3 (K.K.)** a girl was admitted to the hospital at the age of 7 years because of mental deviations. There was a convergent squint, predominately of the left eye. Visual acuity was 3-4/60 on the right eye and 2/60 on the left eye. There was no pigmentation in the macula and attenuated vessels.

**Patient 4 (F.H.)** a girl had noticed visual reduction since the age of 3 years. At about 10 years of age she had noticed poor colour discrimination. Red and blue were confused like blue and white. Her performance at low illumination was poor.

When she was examined at the age of 14 years she could count fingers at the distance of 1 m. There was a constriction of the visual fields in the upper part and a great loss in the central area. There was a greyish spot in the macula surrounded by interrupted stripes and specks of pigment. The fundus appeared pale and the vessels attenuated.

She was mentally reduced at the age of 18 years by which time epileptic fits of the grand type had occurred. The visual acuity was then reduced to light perception with certain projection. But even in this advanced stage she preferred low illumination to daylight.

**Patient 5 (H.S.)** a girl was examined for the first time at the age of 12 years. She had attacks of fury and stuttering from the age of 3½ years and there was a tendency to stumble. There was pigmentation in the fundus of salt and pepper type. Visual acuity varied from 3/60 to 5/60 and was reduced to light perception at 19 years. At 21 years only a 9/60. At 25 years her visual fields had been lost in the central and nasal part at the age of 25½ years.

**Patient 6 (E.B.)** a boy was nearly 7 years when examined. Slight visual reduction had been noticed during a half a year period when he also had been more prone to stumble. Visual acuity was 6/40 on each eye. The visual fields were constricted. Small optic discs and attenuated retinal arteries were found. There was atrophy of the optic nerves and fine mottled pigmentation in the central part of the fundus.

asily confused colours especially red and green and also green and orange signals could not be seen except yellow which was quite easily recognized s were more easily seen with dark and white contrasts than with colour contrasts I clearly reduced vision in twilight though not pronounced At 7½ years of age ar after the first symptoms had appeared the visual acuity was finger counting at distance only in the temporal fields

1 (TE) a boy had noticed reduced vision since the age of 1 years He was nable to discriminate red and green colours The recognition of blue colours was e good The visual acuity was 6/60 on the right eye and 6/36 on the left eye he was examined at the age of 8 years There was some salt and pepper pigment in the central fundus In spite of some uncertainty by the perimetric examination orted good perception in the temporal and lower fields but significant loss in the inatal fields

## Methods

ination of colour vision was performed with the following tests The AO HRR he Farnsworths D 15 test the City University Colour Vision Test the Farns a tritan plate the Farnsworth Munsell 100 Hue test and the Sloan's achromatopsia series of tissue paper contrast charts has been described earlier (Hansen 1960) olour vision tests were administered under two Macbeth Illuminant C lamps (300 A Nagel anomaloscope type I was used for the anomaloscope examinations rmetric examinations during chromatic adaption were performed in a modified nann perimeter as has been described earlier (Hansen 1964a Hansen & Scim Near monochromatic interference filters (half band width 10-15 nm) were used course of dark adaptation was registered with the Goldmann Weekers adapto by the integral adaptation method ERG responses to single flash and flicker li were recorded using an average technique Pulse synchronous variations in ocular pressure were registered by dynamic tonometry as described by Har

## Results

### scopic and scotopic light thresholds

standard static perimetry curve in patient 1 is depressed especially al part (Fig 1) Dark adaptation was repeatedly registered at e of 8 years and showed a slightly flattened curve in the first a e the final thresholds being about 1 log unit above the normal e sensitivity during total dark adaptation shows a typical rhod 4) Static perimetry under scotopic conditions (Fig 2) demon moderate increase of absolute light thresholds in the central ed with the normal values differing by a little less than 1 r from about 25 in the periphery the light thresholds are

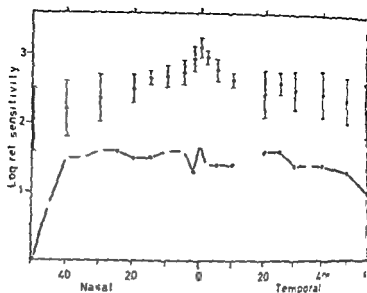


Fig. 1

Static perimetry in standard illumination ( $10 \text{ cd/m}^2$ ) registered in patient 1. Size of target 54 (object IV). Mean  $\pm 2$  SD for 5 normals is indicated.

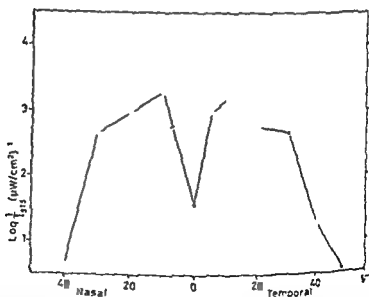


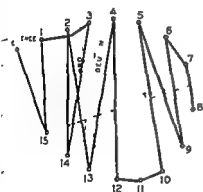
Fig. 2

Static perimetry during total dark adaptation recorded with green object ( $\lambda = 515 \text{ nm}$ ). Ordinate: absolute threshold sensitivity for target subtending  $2^\circ$  diameter. Shaded area indicates the variation (mean  $\pm 2$  SD) for 5 normals.

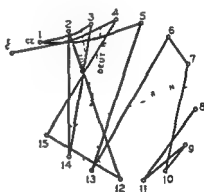
sed Dark adaptation registered in patient 7 at the age of 8 years showed a  
 erate flattening of the curve the final thresholds being about 2 log units  
 e the normal level Patient 4 who was examined at the Eye Department  
 vål Hospital Oslo had dark adaptation curves at about the normal level

ur vision

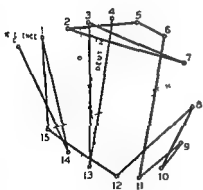
performance of three patients with the Farnsworth s D 15 test is shown in  
 3 The confusions of patient 1 (CC) were mainly along the protan axis (re  
 ured at the age of 1 years) Later on the confusion axes were more irregular



CASE 1



CASE 6



CASE 7

*Fig 3*

Performance of the Farnsworth s D 15 test for 3 patients



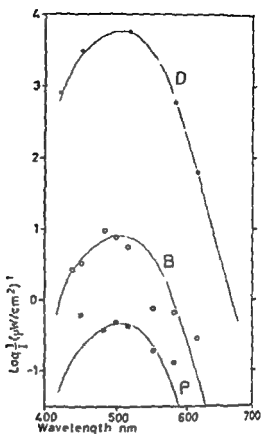


Fig 4

Spectral threshold sensitivity during total dark adaptation (D) in blue violet background (B)  $\lambda_{max} = 421$  nm 3 lux and in purple background (P) Wratten 54A 3 lux records are 10 nasal central and 25 temporal for the three conditions respectively and angular size of targets 34 1°47 and 1.4° respectively Fitted sensitivity curves (after the ICE standard) are indicated.

though clearly of red green type Patient 6 (FB) also showed an irregular pattern though with dominance of confusions along the red green axis. A red green defect was more clearly shown with the City University test where protan or deutan alternatives were preferred Patient 7 (TF) concerned only and had irregular confusions showing some preponderance along the green axis The F D 15 test could not be performed by patient 4 due to reduced visual acuity like patient 2 who could only see coarse grey shades

With the OA HRR test patient 1 could see all the figures on the test charts except on the screening charts and only one of the red error

4 the tissue paper contrast charts she was able to read the figures on the 11 with blue yellow green and purple backgrounds in spite of her reduced acuity. On the other hand she could see none of the figures with green or red purple backgrounds. Using the Farnsworth's tritan chart she could see the green and not the blue square. She had 11 error scores on the 100 Hue showing an irregular pattern though with less error scores in the red region.

With the anomaloscope examination she could obtain matches over the entire scale. However the extreme red could not be seen. Other red qualities were matched with dark yellow (10 on the red green screw with 3-5 and 68-15). Extreme green was just brighter than the brightest yellow. With the Munsell achromatopsia test red could be matched with a dark grey (3.5) while a match could be obtained for the other colours.

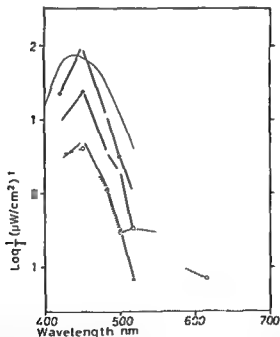


Fig 3

Spectral threshold sensitivity in yellow adapting light (Wratten 91 2300 lux at 7° nasal position (lower curve) compared with that of a normal person (age 21) obtained centrally (stippled line). The upper and middle curves are obtained under reduced illumination (500 lux) at 7° nasal and 3° temporal positions respectively. The curves are displaced upwards and separated by 1/2 log unit. The smooth curve indicates the action spectrum of the blue mechanism (after Walraven 1944). Angular size of target 54

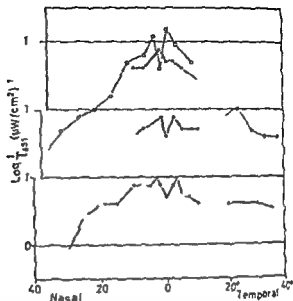


Fig 6

Static perimetry in yellow adapting light (Wratten 21) of varying intensities: (top) 1150 lux (middle) and 800 lux (bottom). Blue target ( $\lambda_{max} = 451 \text{ nm}$ ) at  $34^\circ$  visual angle is used. Stippled line indicates the performance of a normal (age 21).

### Selective chromatic adaptation

In the purple adapting light favouring the green cone response pattern, the patient could not see any targets. However, at  $25^\circ$  peripheral position she could see 6 targets in reduced illumination (50 lux instead of 200 lux). Her response curve corresponds to the rhodopsin curve with an additional peak in the periphery which might be a contribution from the blue mechanism (Fig 4). In the background, a rhodopsin response curve is obtained by central fixation. Thus, during total dark adaptation as well as in the adapting purple light, her response curve is dominated by receptors of the rhodopsin type.

In the yellow adapting light ( $\lambda > 530 \text{ nm}$ ) the patient's vision was extremely poor in spite of the high illuminance value of 2300 lux. Consequently, very large fixation marks had to be used. The spectral peak sensitivity in the yellow light was at about 451 nm, showing a good blue cone response. Static perimetry performed in yellow light of varying intensities with the same target shows a good threshold sensitivity even in the central part at the same level as in a normal person (Fig 6).

ient 6 (at the age of 6 years) could clearly see a blue target ( $\lambda_{\text{max}} = 440 \text{ nm}$ ) against a yellow background (Na light at  $\lambda = 589 \text{ nm}$  1300 lux) in the central visual field. However, it was not possible to reliably register light thresholds. By standard perimetry this patient was not able to see any red target. Another patient (7) indicated that he could not see any targets against a white adapting field.

#### Electroretinography

Patient 1 ERG was present at the age of 6 years. Reduced amplitudes, especially low b waves, were registered with single flash stimuli under scotopic conditions. Flicker registration (40 Hz) showed the same size of amplitudes, although with alternating peaks. ERG was registered also at the Moorfields Eye Hospital, London, at the age of 7 years and showed reduced amplitudes. At the



*Fig 7*

Fluorescein angiogram of patient 1 at the age of 7 years

age of 8 years the ERG was extinguished. Patient 3 showed no response under photopic conditions nor to slow flash ERG. Patient 4 ERG was extinguished at the age of 14 years.

### Fluorescein angiography

The fluorescein angiogram obtained in patient 1 at the age of 12 years showed moderate well limited fluorescence comprising an elliptic area in the central region (about 10° diameter). The fovea itself is a normal dark spot. No sign of leakage was registered in the present series of pictures.

### Dynamic tonometry

Corneal indentation pulse amplitudes were registered in four patients. The amplitudes measured 20  $\mu$  in patient 1, 46  $\mu$  in patient 2, 34  $\mu$  in patient 3, and 22–20  $\mu$  in patient 6. The patients were examined at the ages of 12, 13, 14 and 17 years respectively. The results are within the normal values found by Schiøtz (1950) for the same age groups.

### Other findings

Vacuolized lymphocytes in the blood were found in high numbers in all patients, varying from 10% to nearly all of the lymphocytes being affected.

EEG was normal in patient 3 and in patient 1 in her first examination. Later on slight irregularities were shown. Irregularities indicating cerebral affection were found in the others.

The initial neurological examinations revealed no pathological changes except in patient 3 where instability of the lower extremities and a slight clonus of the muscles were found.

## Discussion

In familial cerebro macular degeneration a fundamental failure in the oxidation metabolic inactivation which most likely affects certain systems relatively early during the course of the disease. Affected patients lose their retinal rods and cones (Zeman 1974). Characteristically the disease debuts with complaints related to central vision. The age of onset in our patients was 3 to 10 years. Visual disturbance was the only initial symptom in patients 3 and 6 where slight mental deviations had also occurred.

The degeneration of the neuro epithelium appears to be of the same type as the histopathological examinations have always shown to be. The normal values found by

of corneal indentation pulse amplitudes in our four patients indicate a good ocular circulation and is in accordance with this observation. Apparently the degeneration of the neuro epithelium also involves the pigment epithelium as the fluorescein angiogram registered in patient 1 suggests a distinct defect of the pigment epithelium in the macular region. There is probably no defect of the Bruch's membrane as no leakage has been observed. The absence of ERG response in patient 3 as early as at 1 year of age and in patient 4 at the age of 14 years indicates a great disorder of the outer retinal layers. Copenhagen & Goodman (1960) found absent or markedly depressed ERG in juvenile amaurotic family idiocy as opposed to a normal or borderline ERG in infantile amaurotic family idiocy. In patient 1 ERG was present though reduced at the age of 7 years. At this early stage there was also flicker response which suggests functioning cones. Most probably this means blue cone activity, possibly activity of peripheral cones. However already after one year the ERG was absent.

A characteristic feature in our patients was the rapid progression of the visual failure in many cases leading to practical blindness within a few years after onset of the disease. Rather than a general constriction of the visual fields a typical finding in our patients was the considerable loss in the central area in advanced stages as was seen in patient 3 and 6. Loss of the nasal field may also occur.

As a curiosity of this kind of visual field loss a binasal hemiopia led to a misinterpretation of the disease by Daas who in 1869 reported six cases which evidently were familial cerebro macular degeneration. Despite his description of typical retinal changes he concluded that the disease must be related to affections of the visual pathways and not to the retinal changes.

Colour vision has not usually been referred to in reports of familial cerebro macular degeneration probably because of the great visual reduction in those patients. Oatman (1911) found central scotoma for green and red as one of the earliest manifestations. Copenhagen & Goodman (1960) in one patient found impaired colour discrimination which could not be classified. In a report of Ozin et al (1962) of adult hereditary cerebro macular degeneration severe colour blindness was found in one patient a male having considerable visual loss and a further deterioration of vision in bright light. The type of the colour defect was not specified.

Typical red green colour defects were found in our patients. The defects were pronounced already in the early stages. Patient 1 evidently had an acquired red

green defect type I (after Verriest 1964) By the anomaloscope two matches were of achromatic type suggesting no red or green receptor function confirmed by the chromatic adaptation experiments Only action spectrum rhodopsin type were obtained demonstrating here an example of a type of vision (Fig. 3) It is to be noticed that this patient was markedly stimulated in yellow illumination containing only long wavelength light.

A remarkable finding was the good response of the blue cone mechanism as was seen with patient 1 (Fig. 4 and 5) The good perception of blue was confirmed by the colour vision tests Likewise in patients 6 and 7 a good perception of blue colours had been noticed.

The retinal affection found in familial cerebro macular degeneration is different from that of retinitis pigmentosa where the blue mechanism is often more greatly reduced (Hansen 1977) There is also a clear difference concerning night vision which in retinitis pigmentosa is typically lost at an early stage. A relatively good night vision was indicated by our patients even in advanced stages of the disease This is consistent with observations referred by De Vries (1911) in his survey including typical cases of maculocerebral degeneration.

Differential diagnosis may be difficult in the case of Stargardt's disease which in fact is a central cone dystrophy In Stargardt's disease the typical fundus finding is a central white spot likewise a colour vision defect of the red green type (François et al 1964) A distinct blue receptor response may also be shown (Hansen 1974b).

Among familial cerebro macular degenerations individual differences may occur However in our patients representing a common type in which the degeneration is chiefly central a selectivity was shown as to the function of the receptor functions It is difficult to explain why the blue mechanism is better preserved than the red and green mechanisms It may be that a more extensive damage over a wider area of the retina is necessary for the blue receptor mechanism to be lost Besides the primary degeneration taking place in the receptor cells a secondary degeneration is also in progress in the ganglion cells that is in the optic nerve elements The selective affection of the receptor responses probably can be explained on the basis of both types of degeneration.

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## SCREENING OF RED GREEN DEFECTS OF COLOUR VISION WITH PSEUDOISOCROMATIC TESTS

BY

EERO AARNISALO

Fifty red green defectives and 100 normal subjects were examined with the second edition of the Bostrom Kugelberg (BK II 1972) series and the Ishihara complete edition (1976) of pseudoisochromatic plates. The results are related to those obtained with the first edition of the Bostrom Kugelberg test (BK I 1944) and the Bostrom test (II B 1950) and to the classification of defects obtained with the Nagel anomaloscope.

The 50 red green defectives were originally selected by using a combination of the BK I and II tests. The normal subjects also passed this preliminary test as well as an examination with the Nagel anomaloscope.

In the final examinations performed under standardized conditions three red green defectives passed both the BK II and the BK I test while eight defectives passed the Ishihara test. Combination of BK II or BK I test with the Ishihara test does not improve the result. Only one defective (a borderline case of protanomaly) passed the separate II test.

Normal subjects were not classified as colour defectives with any of the four pseudoisochromatic tests used. All normal subjects passed both the BK II and the Ishihara test. Classified as suspected red green defectives (one misreading made in standardized conditions) were five normal subjects with the II test and one normal subject with the BK I test.

In the second edition of Bostrom Kugelberg series the plates numbered 3, 5, 11, 16 and 18 are clearly less effective than respective plates of the first edition. Only the plates numbered 1 and 10 have markedly improved in the second edition.

*Key words:* colour vision screening - pseudoisochromatic plates - red green defectives

Red green defectives made on average 0.54 misreadings per figure in the BK II test as compared with respective 0.67 in BK I 0.69 in the I I test and 0.56 in the II II test

The first edition of the Bostrom Kugelberg pseudochromatic test (1944) and the second edition (BK II 1972) both consist of 39 plates of vanishing type showing figures of numbers (15 plates) or serpent line plates) or no figure at all (3 dissimulation plates). According to instructions of this test persons making one misreading are suspected of defective red green colour vision two misreadings indicate defective red green vision while persons with normal colour vision are likely to pass the test without misreadings. The BK I series is considered effective in detecting red green defects (Kugelberg 1948 Chapanis 1948 Hettessy 1955 Belcher & Hansen 1963 Dreyer 1969) but some anomalous trichromats may pass the test without misreadings (Kugelberg 1948 Belcher et al 1958 Dreyer 1969). Moreover the plates of the BK II test may be read correctly by some anomalous trichromats (Hedjn 1974).

The Ishihara test was first published in 1917. The latest two editions are the complete series (38 plates 1976) and the smaller series (24 plates 1976). The plates of the complete series consist of two demonstration plates, 11 plates with a number figure (9 plates of vanishing type 8 plates of transformation type 4 hidden digit plates and 4 diagnostic plates) and 13 plates with a serpent line. According to the instructions for this test the first series is used to differentiate between normal and defective colour vision. Persons making five to seven misreadings are suspected of having defective red green colour vision eight or more misreadings indicate defective colour vision. Normals are likely to make maximally four misreadings. There are many reports on effective differentiation between normals and red green defectives obtained with the Ishihara test (Sloan & Habel 1956 Belcher et al 1958 Crone 1961). Allowing 40 per cent of plates misread by normals, the differentiation between normals and red green defectives was obtained in the fifth (1925) seventh (1936) and ninth (1940) edition (Hardy et al 1951) tenth (1951) edition (Katajisto 1961) and eleventh (1954) edition (Hettessy 1955) however found the tenth edition to be less effective. Some anomalous trichromats have been found to pass the Ishihara test (Habel 1956 Belcher et al 1958 Dreyer 1969).

The Bostrom (II B 1950) test consists of 16 plates 15 of them showing figures of numbers the last one being a dissimulation plate. The first series of this series is used only for demonstration thus only 14 plates of the vanishing type can be used for detecting red green defects. According to the

of this test persons making one misreading are suspected of having defective red green colour vision two misreadings indicate defective colour vision while persons with normal colour vision are likely to pass this test without misreadings. The II B series is considered to detect red green defects effectively (Belcher et al 1958 Dreyer 1969).

The purpose of the present study was to investigate the effectivity of the 2nd edition (1972) of the Bostrom Kugelberg test and the last edition (1976) of the complete series of the Ishihara test in screening of red green defects of colour vision and to compare the results with those obtained with the first edition (1944) of the Bostrom Kugelberg series and the Bostrom (1950) test as well as with the classification of defects obtained with the Nagel anomaloscope.

## Material and Methods

Red green defective subjects were selected by testing 351 male students at the university with plates of the Bk I and II B series in combination. The tests were shown in diffuse indirect midday daylight and subjects making one or more misreadings were chosen for further examination. These included tests in the following order with all plates of the Bk I II B and Bk II tests and with the first 25 plates of the Ishihara (1946) complete series. Thus in the Ishihara test the plates numbered 26 to 38 (serpent lines) were not shown. The tests with pseudoisochromatic plates were performed with a Macbeth reflective (BBX 324) daylight illuminator (effective illumination 1850 lux, colour temperature 7500 K). According to the instructions of the respective tests the plates of the Ishihara series were exposed for four seconds and the plates of the other tests were exposed for 15 sec. All misread plates were shown a second time. Next followed an examination with the Nagel anomaloscope including measurements of the mid matching point and the matching range. The n range (the eye conditioned to the white light field of the anomaloscope) and the u range (the eye conditioned to the bipartite coloured field of the anomaloscope) were also measured (Schmidt 1955).

In the final series of 50 red green defective subjects (young men aged 19-36) the quotient of anomaly based on the mid matching point of the n range varied between  $\leq 0.10$  and  $\geq 2.0$ . An u range 10 scale units larger than the n range was chosen to define extreme anomaly.

All colour vision tests except the anomaloscope examination were performed binocularly. The subjects used their regular eye glasses only all types of tinted glasses were excluded. Subjects with myopia higher than 4 D were excluded. There were subjects with pathology of the eye fundi or a corrected visual acuity below 1.0.

In the series of 100 normal young men (pupils of navigators 18 to 31) the quotient of anomaly was within normal limits 0.72 and 1.28. The normal subjects passed all examinations as described.

## Results

Table I shows the number of plates misread by normal and red green defective subjects in the four different pseudoisochromatic tests mentioned above. In the Bk II series normal subjects made no misreadings and three red green defectives passed the test while two defectives both made one misreading. In the Bk I series one normal subject made one misreading three red green defectives passed the test and one defective made one misreading. Of the 100 normal subjects made no misreadings in the Ishihara test, 67 normals made one misreading three made two one made three and one normal subject made four misreadings. All red green defectives made misreadings in the Ishihara test two of them made only one misreading made two and four defectives made three misreadings. Eight red green defective subjects thus passed the Ishihara test. Five normal subjects made misreading in the II B test but only one red green defective subject passed this test without misreadings.

Table II illustrates the effectivity in detecting red green defects of separate plates of the four pseudoisochromatic tests. In Bk II series plates numbered 6 and 10 are quite good. Normal subjects made no misreadings and only nine red green defectives (plate number 6) and respectively defectives (plate number 10) read these plates correctly. Less good are plates numbered 2, 5 and 11 as shown by the fact that a high number of defectives 49, 45 and 44 respectively were able to read these plates correctly. In the series the plate number 2 also has a low effectivity. All normal subjects and 48 red green defective subjects read this plate correctly.

The best plates of the Ishihara test are those numbered 6 and 9. All normal subjects read these plates correctly but only seven red green defectives (plate number 6) and nine defectives (plate number 9) read them correctly. That as many as 28 normal subjects were unable to read correctly the plate number 17 on the other hand no red green defective subjects was able to read this plate correctly. Of an opposite kind is the effect of plate number 11. Only one normal subject misread this plate but 19 defectives read it correctly.

In the II B test one normal subject misread the plate number 5 but on the other hand only two red green defectives read it correctly. Plate number 1 is less good all normals passed but also 44 defectives read it correctly.

Table 1

Number of plates misread in tests of Bostrom Kugelberg second edition (1972) Ishihara complete series (1976) Bostrom Kugelberg first edition (1944) and II Bostrom series (1950) by 100 normal subjects and 50 red green defectives

		Number of plates misread																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BK II 1972	normals	100																					
	defectives	3	9	2	2	1	4		1	2	4	6	6	7	8								
Ishihara 1976	normals	67	94	3	1	1																	
	defectives	2	2	4	2			1	1	1	1	2	1			5	2	6	9	6	6		
BK I 1944	normals	99	1																				
	defectives	3	1	2	3	1	1	1	1	1	3	1	2	5	8	3	3	1					
IIB 1950	normals	92	5																				
	defectives	1	2	3	2	1	5	7	4	9	7	4	3	2									

Table II

Number of normal subjects misreading and number of red green defectives reading correctly the separate plates (serial number indicated) of tests of Bostrom Kugelberg second edition (1972) Ishihara complete series (1976) Bostrom Kugelberg first edition (1944) and II Bostrom series (1950) Results obtained by 100 normal subjects and 50 red green defectives are shown

		Plate number in each series																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BK II 19/2	normals defectives	90	47	19	25	45	9			12	5	44	14	24	19		22	17	33	15	16	
Ishihara 19/2	normals defectives	10	25	18	15	7	11	9	6	16	12	14	14	18	15	14	10	0	13	24	28	39
BK I 144	normals defectives	34	48	11	19	35	11			13	18	27	11	2	12		10	10	16	18	17	
HB 19/2	normals defectives	90	99	11	27	95	33	1	15	15	15	16	11	44								





Red green defectives made in average 9.1 misreadings 0.34 per separate plate in a total number of 17 plates (dissimulation plates of the Bk II test as compared with respective 10.5 and 0.67 in the 13.9 and 0.69 in the 20 screening plates numbered 9 to 21 (demonstrative and serpent line plates excluded) of the Ishihara test and 0.56 in the 14 plates (demonstration and dissimulation plates excluded) of the B test.

The number of misreadings made in the four pseudoisochromatic different types of red green defectives classified with the Nagel test are shown in Table III. Note that some subjects with deuteranomaly made no misreadings or only a few misreadings while one subject with extreme anomaly and no subject with dichromacy gave a low number of misreadings.

Also the diagnostic plates numbered 22 to 29 of the Ishihara test were read correctly by both the normal subjects and the red green defectives of the present series. Of the 50 red green defectives two or more misreadings in the diagnostic plates in these 37 cases (74 per cent) qualitative diagnosis was correct in the rest of 13 cases the diagnosis was not possible. Of the anomalous trichromats of the present series qualitative diagnosis was possible and correct in 11 cases but quantitatively all 16 cases were classified as severe. The matching range obtained with the anomaloscope (in range) was 4 scale units in one of these cases, 4 scale units in two cases and less than 4 scale units in the rest of 13 cases. Obviously the quantitative diagnosis obtained with the anomaloscope is unreliable and in many cases qualitative diagnosis cannot be made.

## Discussion

Proper use of a combination of the Bk I and II B series of pseudoisochromatic tests provides an effective preliminary screening of red green defective colour vision (Chapanis 1949, Irev 1958, Katavisto 1961). Using this method in the screenings of the present study a high number (50) of red green defective persons were collected by examining 351 persons. A relatively high rate (11 per cent) of red green defectives in this population may partly be due to the fact that persons previously suspected to have defective colour vision may be more likely to volunteer for the examinations. On the other hand some anomalous subjects may pass an examination with pseudoisochromatic tests (Lemberg 1969). In the present series the deutan/protan ratio of red green defective subjects was 5.25 as compared with 4.91 in a population examined in Finland with the Nagel anomaloscope (Forsius et al. 1968).

the protanomalous subject of the present series was able to read correctly plates of the Bk I and II B series but he performed very slowly and read a full 15 seconds per plate to pass these tests. In the Ishihara test he made one misreading only thus also passing this test. His quotient of anomaly  $Q$  on the mid matching point was 0.10 the  $n$  range being 2 and the  $u$  range 8 scale units (the quotients corresponding to the limits of the  $u$  range 0.59 and 0.91) and his match of pure red to yellow indicated abnormal sensitivity function in the red end of the spectrum. Similar borderline cases of protanomaly have been described (Trendelenburg 1939 Heinsius 1975 p 19/2).

In the present study normal subjects were not falsely classified as red green defectives with the Bk II or Ishihara test. Three red green defectives passed the Bk II test and two were additionally classified as borderline cases. Eight green defectives passed the Ishihara test and three were classified as borderline cases. Reducing the number of misread plates allowed in the Ishihara test from four to three does not alter the result. Only three misreadings are allowed in Sweden in the official use of the Ishihara test (SOSFS/977).

It is recommended in the Ishihara test to note whether the person to be examined reads the hidden digit plates numbered 18 to 21 easier than the plates of vanishing type numbered 10 13 14 and 17. In the present series this procedure did not improve the result obtained with the Ishihara test. The three red green defective subjects who passed the Bk II and Bk I tests also passed the Ishihara test. The eight defective subjects who passed the Ishihara test all showed a narrow ( $n$  range less than 5 scale units) and anomalous matching range but an abnormal quotient. In a large series of red green defective subjects there is a correlation between the number of errors made in pigment tests and the matching range obtained with the spectral matching of the anomaloscope but this may not be true in individual cases (Heinsius 1979). One of the deuteranomalous subjects of the present series misread 13 plates in both Bk II and Bk I tests 18 plates in the Ishihara test and 14 plates in the II B test but showed a matching range ( $n$  range) of 1 scale unit only. On the other hand one red green defective subject who passed the Ishihara test showed in the anomaloscope examination a  $n$  range of one scale unit but a  $u$  range of 16 scale units and was classified as a case of extreme anomaly. He made three misreadings in both Bk II and Ishihara tests and seven misreadings in both Bk I and II B tests. Plates numbered 2 5 and 11 of the Bk II series have low effectivity. Only one red green defective subject misread the plate number 2 of the Bk II series and this was a case of extreme protanomaly making a total number of 13 mis-

readings in this test. In the second edition of the Bostrom test the plates numbered 3, 5, 11, 16 and 18 are less effective than plates in the first edition of this test. Only the plates numbered 1 and 2 in the second edition have markedly improved. Also the average number of misreadings as well as the number of misreadings per separate test by red-green defective subjects is lower in the Bk II series as compared with the Bk I series. In Bk I series the coloured spots forming the test figures and the spots of the background are printed on a slightly coloured background of the same hue as the spots of the background, while the colours of the test figures in the Bk II series are printed on a uniformly white background. Saturation of the colours in the Bk II series thus appears to be somewhat higher than in Bk I series.

The most effective separate plate of the four pseudoisochromatic tests appears to be the plate number 8 of the II B series. One normal subject failed to read this plate but only two red-green defectives (one case of deuteranomaly and one of protanomaly) passed. Two deuteranomalous subjects who passed the Bk I test and Bk I tests both misread the plate number 8 of the II B test. One of them misread the plate number 13 and one of them misread the plate number 10. The II B test may be somewhat difficult for normal subjects (Hietanen 1955, Frey 1958, Katavisto 1961, Heinis 1963). In the present study five normal subjects (5 per cent) failed in this test (only one failed if plate number 12 is excluded).

Screening of red-green defects of colour vision is usually made with a combination of two reliable pseudoisochromatic tests (Frey 1958, Heinis 1963). In the present series of colour defective subjects combination of the Bk II or Bk I test with those obtained with the Ishihara test does not improve the result obtained with separate Bk II or Bk I test as the three red-green defective subjects who passed both these tests also passed the Ishihara test. The separate II B test is more effective than the Bk II test; only one red-green defective subject passed. This was the borderline case of protanomaly described above.

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facility. The reason is that the possibilities for surgical and uveoscleral pathways have not been investigated in any detail. This describes the results of our experimental study on the surgical and uveoscleral outflow.

## Material and Methods

Six series of experiments were performed on 43 isolated cadaver eyes 24-36 h after death. In all cases death was sudden. In the first series we measured the scleral outflow facility; in the others five surgical techniques for evaluating the uveoscleral outflow were evaluated.

**Scleral outflow facility measurement.** A needle (inner diameter 1 mm) was inserted through the optic nerve into the eye. The vitreous body was removed by vacuum suction. The lens, the retina and the uvea were destroyed and separated from the eyeball by the sharp end of a needle and a tiny hook introduced through the needle. With the help of the latter the large pieces of tissue were removed from the eye. The tissue remnants were removed by irrigation and suction. The preparation was connected to a perfusion system which was filled with isotonic salt solution.

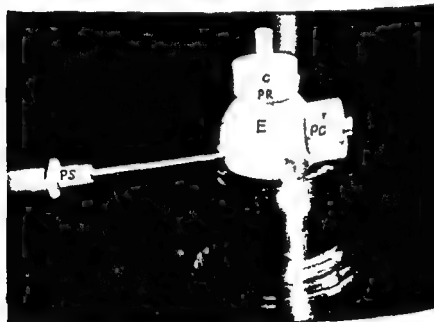


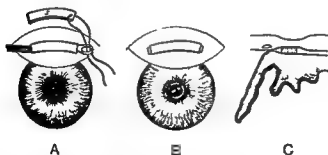
Fig. 1

Scleral outflow facility measurements. A needle which connected with perfusion (PS) is inserted into the eyeball (E). A plastic ring (PR) is fixed on the sclera with cyacrin glue and covered with a cover (C). Conventional outflow pathway is realized by a peritubal suction cup (PC).

the system and in the eye was maintained at 20 mmHg. With the help of an operating microscope the area of sclera free of perforating emissaries was found and marked. A plastic ring was placed on this area of sclera and fixed with a synthetic glue (n SO 4). The inner diameter of the ring was 9 mm, the outer diameter 12 mm and the height 9 mm. Conventional outflow pathways were compressed with a perilimbal cup. The inner diameter of the cup was 11 mm, the outer diameter 13 mm and the negative pressure in the cup was 50 mmHg. Then the scleral surface inside the ring was dried with a strip of filter paper and a cover was placed on the plastic ring in order to eliminate evaporation of the fluid (Fig 1). After 15 min the ring was opened and the fluid which had gathered in the ring was removed and measured with the help of a micropipet. The procedure was repeated with the pressures in the eye equal to 30 mmHg and 40 mmHg. The measurements were made in seven eyes.

#### *Implantation of a scleral strip into suprauveal space*

A hypodermic needle connected to the perfusion system was introduced through the sclera first into the posterior chamber and then through the pupil into the anterior chamber. The needle touching the margin of the pupil prevents deepening of the anterior chamber during perfusion. The outflow facility was measured at a pressure of 20 mmHg. Two radial incisions in the sclera were performed 3 to 5 mm from the corneal limbus. The distance between the incisions was 10 mm (Fig 2). A spatula with an opening at the end was introduced through one incision into the suprauveal space and the other end of the spatula was passed through the other incision. A strip of sclera 10 mm long and 2 mm wide and about 0.5 mm thick was excised from another eye and one end of the strip was sutured. The ends of the suture were introduced into the opening at the end of the spatula and tied. The spatula was withdrawn and the scleral strip was pulled into the suprauveal space. The strip was placed parallel to the limbus approximately



*Fig 2*

#### *Implantation of a scleral strip into suprauveal space*

A spatula is introduced into the suprauveal space through the radial scleral incision and the end of the spatula is taken off through the other incision. A strip of sclera (SS) is excised from another eye and one end is sutured. The ends of the suture are threaded through the eye at the end of the spatula.

The scleral strip is placed into the suprauveal space.

The anterior part of the suprauveal space is dilated with the scleral strip.



3 to 5 mm behind it. The radial incisions of the sclera were closed with glue. The outflow facility was measured for 10 min. Eleven enucleated eyes were studied.

**Cyclodialysis.** In seven cadaver eyes an incision of sclera 3 mm long and 1 mm from the corneal limbus and parallel to the latter. Through this incision a dialysis for a distance of 5 mm was performed with a spatula. The incision was closed with a suture and the incision was closed with glue. The outflow facility was measured before and after closure of the scleral wound at a constant pressure of 20 mmHg.

**Implantation of a scleral strip into the suprachoidal space combined with cyclodialysis.** The technique of the implantation of the scleral strip into the suprachoidal space was the same as described above. However, before introducing the strip into the suprachoidal space a cyclodialysis was performed for a distance of 5 mm through the radial incisions of the sclera. Five eyes were studied with the help of this technique.

**Implantation of a scleral strip combined with sinusotomy.** After enucleation of the eye a rectoangular limbal based scleral flap 8 mm long and 5 mm wide was prepared. 3) The flap included approximately 2/3 of thickness of the sclera. The deep scleral flap 3 mm long and 3 mm wide was excised and Schlemm's canal was opened. The scleral strip was then introduced into the suprachoidal space as described above. The scleral flap was fixed with sutures and cyanoacrylate glue. The C values were measured before surgery and 10 min after this at a pressure of 20 mmHg. 10 eyes were studied with this technique.

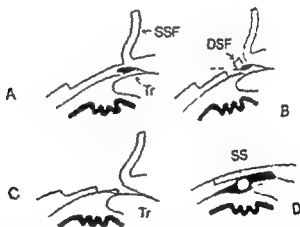


Fig. 3

Implantation of a scleral strip combined with sinusotomy

- Superficial scleral flap (SSF) is prepared. Note the position of Schlemm's canal and the trabecular meshwork (Tr).
- Deep scleral flap (DSF) is prepared. To open Schlemm's canal an incision is made along the dotted line.
- The deep scleral flap is excised and Schlemm's canal is opened.
- The strip of sclera (SS) is placed into the anterior chamber. The superficial scleral flap is fixed with sutures and cyanoacrylate glue.

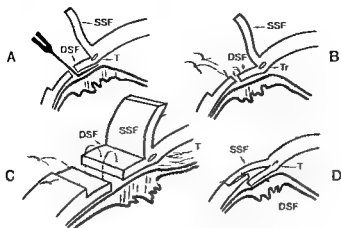


Fig 4

dialysis combined with goniospasty. Superficial (SSF) and deep (DSF) scleral flaps are prepared and cyclodialysis is being made.

and C The anterior lip of the deep scleral incision is sutured with mattress suture both ends of which are passed beneath the posterior lip and taken off at 1 mm behind the incision.

and the incision. The mattress suture is tied up. The superficial scleral flap is fixed with running suture.

*Cyclodialysis combined with goniospasty* The procedure performed on six cadaver eyes as follows. The eye was connected with the perfusion system. A lamellar limbal scleral flap 6 mm long and 3 mm wide was prepared (Fig 4). An incision of the scleral lamina at a distance of 4 mm from the corneal limbus and parallel to the r was made. Cyclodialysis 3 mm long was then performed with a spatula. The anterior lip of the deep scleral incision was sutured with mattress suture both ends of which were passed beneath the posterior lip and passed through at 1 mm behind the incision. At each side of the latter an additional incision of the deep scleral lamina was made. They were directed towards the scleral spur. The deep scleral flap thus outlined was separated from the ciliary body. With the help of the mattress suture a portion of the deep scleral flap was pulled into the suprauveal space beneath the lip of the deep scleral incision. The suture was then tied. The external scleral flap was fixed with running suture and completely closed with the glue. The C values were measured before and 10 min after surgery.

## Results

*Uveoscleral outflow facility measurements* For 15 min the mean amount of fluid which flowed into the plastic ring was equal to 0.63 mm<sup>3</sup> (range 0.23 to 1.3 mm<sup>3</sup>) at a pressure of 20 mmHg. 0.98 mm<sup>3</sup> (range 0.30 to 2.0 mm<sup>3</sup>) at a pressure of 30 mmHg.

Table 1

Surgical stimulation of the uveoscleral outflow in enucleated eyes (mm<sup>3</sup>/min/mmHg) before and after surgery

Techniques	No of eyes	Initial C values		Final C values	
		Mean	Range	Mean	Range
Implantation of scleral strip into suprauveal space	11	0.151	0.11-0.31	0.222	0.14-0.41
Cyclodialysis	7	0.134	0.11-0.23	0.200	0.13-0.32
Cyclodialysis + implantation of scleral strip	3	0.171	0.07-0.30	0.244	0.11-0.31
Sinusotomy + implantation of scleral strip	10	0.143	0.12-0.22	0.244	0.12-0.31
Cyclodialysis + goniospasy	5	0.160	0.13-0.21	0.260	0.13-0.33

The pressure in the perfusion system was 20 mmHg and the temperature of the perfusion solution was 20°C.

pressure of 30 mmHg and 1.40 mm<sup>3</sup> (range 0.34 to 3.1 mm<sup>3</sup>) at a pressure of 20 mmHg. The area inside of ring was equal to 63.6 mm<sup>2</sup>. One can calculate the mean outflow facility coefficient was pressure independent and equal to 0.0036 mm<sup>3</sup> min/mmHg per 1 cm (100 mm<sup>2</sup>) of scleral surface.

*Implantation of scleral strip into suprauveal space.* The results are shown in Table 1. After surgery the mean C value increased by 0.011 ± 0.007 mmHg (mean ± SEM). This difference is significant ( $P < 0.05$ ). The increase in the C value was 23%, range 7 to 35%. After removal of the strip from the suprauveal space the C value returned to the initial level or even lower value.

*Cyclodialysis.* The mean increase in the outflow facility coefficient was 0.015 mm<sup>3</sup> min/mmHg. The C value increment varied from 1 to 35% averaging 30%.

*Implantation of a scleral strip combined with cyclodialysis.* The results are shown in Table 1. The mean increase in the C value was 0.103 mm<sup>3</sup> min/mmHg (range 0.02-0.13) i.e., 23%.

to the sum of the mean C values increments in two previous series of experiments C value increased in each case by 24 %–96 % (mean 60 %) *implantation of scleral strip combined with sinusotomy* After surgery the mean C value increased by  $0.071 \pm 0.014$  mm<sup>3</sup>/min/mmHg The C value increment varied from 18 to 111 % averaging 41 %

*cyclodialysis combined with goniosynthesis* In each of five cases the coefficient of outflow facility increased by 0.06 to 0.14 (mean 0.10) mm<sup>3</sup>/min/mmHg As compared to its initial level the C value increment varied from 43 % to 108 % (mean 67 %)

## Discussion

The uveo scleral outflow route consists of three parts: the anterior portion of the ciliary body, the supraciliary space and the sclera. Each part exerts a certain resistance to the aqueous outflow.

It follows from the first series of our experiments that the rate of flow through the sclera is directly proportional to the outflow pressure. If the latter is equal to the average normal intraocular pressure (15 mmHg) and the whole anterior scleral surface (1400 mm<sup>2</sup>) is available for the aqueous 30 % to 40 % of the outflow (2 mm<sup>3</sup>/min) passes through the sclera. It is approximately twice as much as was reported by Fatt & Hedbys (1940). We admit that in our experiments tiny emissaries could be presented in the areas of sclera under investigation. Taking into consideration the real existence of the perivascular space in the sclera, it is supposed that our data are not far from reality. However, it was demonstrated by Bill & Phillips (1941) that the uveo scleral route is responsible for only 4 % and 14 % respectively of the total outflow in human eyes.

Thus a considerable part of the uveo scleral outflow resistance seems to be located in the uveal and supraciliary portions of the uveo scleral pathways. The resistance of the first portion can be diminished by cyclodialysis and the second portions with the help of a widening of the supraciliary space. The latter appears to be only a potential space. It was found that there is a considerable resistance both to circumferential and meridional flow of fluid in the supraciliary space (Cherkasova 1946).

To widen the supraciliary space a strip of sclera was placed in the anterior portion of the space. Both cyclodialysis and implantation of a scleral strip gave approximately the same results: the mean C value increased by 0.04–0.14 mm<sup>3</sup>/min/mmHg. Cyclodialysis combined with implantation of a scleral strip resulted in a significantly larger increase of the outflow facility coef-

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# TIMOLOL TRANSITORY MANIFESTATIONS OF DRY EYES IN LONG TERM TREATMENT

BY

NIELS VESTI NIELSEN AND JENS SINDBERG ERKENS

In 64 patients treated with timolol eye drops (0.25% and 0.1%) 10 patients developed transitory sensation of dry eyes. Two of these patients also had xerostomia. Conjunctival and corneal defects were observed simultaneously with rose bengal staining. Morphologically some of the lesions might have the same appearance as of the early stages of keratoconjunctivitis sicca. A reduction of Schirmer test and break up time was noted. The duration of symptoms ranged from 3 to 13 days. The time of treatment at the debut of symptoms was 30 weeks (range 10-40 weeks). A pathogenesis of these seemingly harmless findings is at present obscure.

In none of our patients was the treatment discontinued and the symptoms did not reappear.

**Key words:** timolol - adverse effect - transitory - dry eyes - rose bengal staining dots

Timolol maleate, a non selective beta blocker, has been demonstrated to have a potent hypotensive effect on an elevated intraocular pressure when used topically (Katz et al 1976, Kertt & Horven 1978, Kertt & Nielsen 1978, Radius et al 1978, Ritch et al 1978, Zimmerman & Kertt 1978). The future role of timolol in the treatment of glaucoma is at present time to be very promising.

To day no local or systemic significant adverse effects in the long term use of timolol ophthalmic solution have been reported.

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11 complaints (transient burning smarting pain and slightly blurred vision) been noted previously (Nielsen 1948 Radius 1948 Preclinical Brochure timolol 1971)

12 pinctolol and oxprenolol have induced serious ocular adverse effects - stomucocutaneous syndrome - when administered systemically (Felix et al 1971 Garner & Rahi 1976 Holt & Waddington 1975 Wright 1975)

13 In a long term study we have observed transitory superficial lesions of the conjunctiva and cornea together with the sensation of dry eyes in seven patients treated with timolol eye drops

### Materials and Methods

The material consisted of 64 patients 33 women and 27 men, with an increased IOP mean age was 66.8 years (range 30-85 years) Ten were treated with timolol eye drops 0.25% x 1 and 54 with 0.50% x 1-2 The subjects were regularly controlled 1, 3, 5, 9, 13 and 17 weeks from the start of treatment. Thereafter with an interval of 4 weeks

The mean time of observation in the present material was 33 weeks ranging from 1 to 57 weeks

With the exception of one person with rheumatoid arthritis no patients suffered from any form of collagenosis

At control visits corneal sensitivity with Cochet & Bonnet aesthesiometer (Norm 4) and rose bengal vital staining of the conjunctiva and cornea were recorded usually

Schirmer test I then was investigated (Norm 19.4) When the symptoms of dryness appeared in this material break up time measurement B.U.T.

was compared with 25 subjects with

Thereafter ~~mean~~

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Thereafter measurements of IOP with Goldmanns applanation tonometer the diameter of the pupil pulse rate and blood pressure were performed

Normal levels of haemoglobin leucocytes immunoglobulins (IgG IgM IgA) acute phase proteins Rose Waaler RAT ANF and smooth muscle cell antibody were controlled in the subjects with symptoms and signs of dry eyes compared with 25 only selected timolol treated subjects without symptoms of dry eyes

## Statistical procedure

Statistics were carried out by means of Mann Whitney ranksum test and chi square test.

## Results

In our series seven patients four women and three men appeared to have dry eyes and abnormal vital staining of the conjunctiva and cornea with rose bengal dye Two of these patients moreover complained of xerostomia The



mean age of the patients was 72 years (range 63-85 years). The dry eyes together with slight soreness and xerostomia were caused by the patients. One patient had some complaints of dry eyes before changes before treatment with timolol. The symptoms and changes appeared after a mean treatment period of 30 weeks (range 23-40 weeks). The mean duration of symptoms was seven days (range 3-13 days). Two patients were treated with timolol 0.25%  $\times$  1 and the remaining five patients with 0.50%  $\times$  1-2. The mean IOP was 18.7 mm Hg ( $\pm$  0.46 s.d.) in the right eye and 19.7 mm Hg ( $\pm$  0.73 s.d.) in the left eye. None of these subjects had any of rheumatic disease or any other collagen disease before treatment with timolol.

In one male patient antiglaucoma surgery (trabeculectomy) or had previously been performed.



Fig. 1  
An 80-year-old woman with sicca symptoms. Rosenthal's vital dye staining of the cornea shows late and one confluent staining of the cornea.

## Ocular findings

## Bengal vital staining

rose bengal staining slit lamp examination disclosed numerous staining on the medial and lateral bulbar conjunctiva and in varying degrees of stippling on the cornea (Fig. 1). These changes were mainly localised in the interpalpebral fissure. In the bulbar and tarsal conjunctiva a slight hyperemia was noticed. There was no sign of any intraocular reaction. In the affected eyes only one eye was affected.

**Corneal sensitivity** (Cochet & Bonnet's aesthesiometer diameter 0.12 mm) In patients with dry eyes a slight tendency towards reduced corneal sensitivity was observed (mean reduction 6 mm range 3-10 mm) compared with normal measurements ( $P > 0.05$ ).

**Break up time (BUT)** In the affected eyes the mean BUT was 7.1 seconds (range 5 to 9 seconds). In 23 persons without dry eyes measurement of BUT averaged to be 12.1 seconds (range 5 to 25 seconds). This difference was significant ( $P < 0.01$ ).

Table 1

The distribution of increased serum levels of serum proteins and autoantibodies in seven subjects with and 23 subjects without dry eyes

Serum proteins and autoantibodies	+ Dry eyes (n 7)	- Dry eyes (n 23)
Ig G	1	
Ig A	1	
Ig M		
Orosomucoid		2
Complement (C 3)	3	2
Haptoglobin		1
Transferrin	1	4
Antinuclear antibody		
Smooth cell antibody		
LE cell Test		
Waller Rose		1
R A T		

Significant increase  $P < 0.05$  (Chi square test)

**Schirmer test** As symptoms appeared the mean values of Schirmer test were significantly reduced to 6.1 mm/5 minutes compared to 10.2 mm/5 minutes ( $P < 0.01$ ). Previous values obtained showed mean 11.2 mm/5 minutes. In subjects without dry eyes Schirmer test was mean 14.3 (range 11.5 to 17.5).

**Blood samples** The number of subjects with increased serum levels of IgG are illustrated in Table 1. There was a tendency towards increased serum complement (C3) in the group with dry eyes (43%) compared to subjects without symptoms (9%). It is noted that none of the 10 subjects had positive values of RAT smooth muscle antibody or IgA.

No systemic adverse effects were noted. Pulse rate and blood pressure were unchanged at controls. No change in pupillary size was observed. In the seven subjects did symptoms of dry eyes reappear.

## Discussion

At the present time no other reports dealing with the appearance of dry eyes and superficial lesions of cornea and conjunctiva during treatment with eye drops are available. Laboratory studies have failed to reveal any ocular toxicity with timolol ophthalmic solution (Preclinical Brochure, Tröa 1977). However, transient superficial keratitis has been reported in three subjects without unravelling the mechanism of this manifestation.

In our cases it is noteworthy that the symptoms of dry eyes appeared approximately the same time. The subjects spontaneously complained of dry eyes and simultaneously both functional and morphological signs of decreased lacrimal secretion were disclosed. These manifestations were not only present for a short time. It should be stressed that only one of the subjects had suffered from dry eyes before treatment with timolol. We found no ocular or systemic disease which could explain these probable signs of sicca manifestations. However, our clinical findings need confirmation. We lacked a placebo treated control group and have not observed the elimination of tear proteins.

The pathogenic mechanism responsible for the present manifestations is difficult to unravel. Adverse ocular reactions to ocular anticholinergics have been induced by practolol and oxprenolol when used systemically (C. Waddington 1973; Wright 1975). Furthermore, cutaneous side effects of propranolol have been described (Jensen et al. 1976). Immune mechanisms have been suggested to explain both ocular and cutaneous reactions to these beta adrenergic blocking agents. Amos et al. (1976) have

Garner & Rahi 1976 Jensen et al 1976 Mackie et al 1977 Wright  
 No ocular toxicity of practolol has been reported when administered  
 ally to the eye (Vale & Phillips 1973) In our cases we found a tendency  
 creased levels of serum complement (C 3) but this finding is far from  
 usive Low serum levels of ocular applied timolol have been demonstrated  
 linical Brochure on Timolol 1971) However ocular symptoms have not  
 when timolol has been administered systemically (Mackie et al 1977)  
 the sympathetic nerve supply of the lacrimal gland has not  
 explained (Adler 1970) However it may be suggested  
 blocking agents affect the lacrimal secretion by an  
 pathetic innervation of the gland A local untoward  
 film in some predisposed subjects could be a possible  
 ol induced dry eyes We have not obtained analysis of  
 mmunologic investigations of conjunctiva tear flow fluor  
 m levels of timolol in our cases These procedures will be  
 future if symptoms reappear with the hope of clarifying  
 al manifestations of dry eyes during treatment with timolol  
 n

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# THE LONG TERM HYPOTENSIVE EFFECT OF TIMOLOL MALEATE COMPARED WITH THE EFFECT OF PILOCARPINE IN SIMPLE AND CAPSULAR GLAUCOMA

BY

P J AIRAKSINEN

The long term intraocular pressure (IOP) lowering effect of a  $\beta$  adrenergic blocking agent timolol maleate in topical administration was compared with the effect of pilocarpine on simple and capsular glaucoma by means of diurnal pressure curves during a six month follow up

In simple glaucoma timolol was more effective than pilocarpine in lowering IOP. In the follow up a significant but not marked increase of the IOP was observed. In capsular glaucoma timolol was not effective enough but when it was co administered with miotics the IOP lowering effect was better than with either substance alone.

Timolol induced no accommodative myopia, miosis, reduction of tear flow or other side effects. It increased the outflow facility in simple glaucoma but not in capsular glaucoma. During the trial the anterior chamber depth increased while the corneal thickness remained unchanged.

Four out of the six eyes included in a previous report of secondary glaucoma due to chronic uveitis are still after one year of therapy controlled with timolol.

**Key words:** Anterior chamber depth -  $\beta$  adrenergic blockers - ciliary body - corneal thickness - glaucoma - optic disc perfusion - outflow facility - timolol

Timolol maleate is a specific  $\beta$  adrenergic blocking agent inhibiting both  $\alpha$  and  $\beta$  receptors without intrinsic sympathomimetic or local anaesthetic activity (Hall et al 1975). As an ophthalmic solution timolol had an IOP

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lowering effect of 50% in single dose study (Zimmerman & Ka-  
Kriegels'cin 1978). In ocular hypertensives and open angle glaucoma  
IOP lowering effect with no serious side effects was observed (Richter &  
Boger et al 1978a). After ten weeks of maintenance therapy the effect was  
present (Boger et al 1978b, Nielsen 1978).

The purpose of this study is as a clinical trial to compare the  
hypotensive effect of timolol with the effect of pilocarpine in the treatment of  
simple and capsular glaucoma. The patients served as their own controls.  
Effects of timolol on corneal thickness, anterior chamber depth, pupil  
pupillary diameter, tear secretion, blood pressure and pulse rate were  
followed.

### Material and Methods

The clinical material consisted of 15 patients (6 females, 9 males) aged  
72 years (mean 58.7) who all had elevated IOP with glaucomatous changes in  
the optic disc and/or glaucomatous central visual field defects. In this  
study 10 patients (18 eyes) had been treated for simple glaucoma with  
pilocarpine for 3 to 152 months (mean 54). Five patients (8 eyes) with  
glaucoma had received similar therapy for 12 to 28 months (mean 18).  
In some cases adrenaline or acetazolamide had been added to the therapy.  
The therapy had been stopped prior to this study because of local and systemic side effects.  
A fistulizing operation had been performed on four simple and one capsular  
glaucoma eyes. Three of the fistulas had become obliterated and the eyes  
treated with pilocarpine. One operated simple and one capsular glaucoma  
showed normal IOP and did not take part in the trial. The patients were  
included in this study because they either had an uncontrolled IOP (5 eyes)  
or were inconveniently managed with miotics (3 eyes) or both (14 eyes).

The patients had no history of chronic obstructive pulmonary disease  
or other disorders contraindicating the administration of  $\beta$ -adrenolytic  
agents. An informed consent was obtained from the patients.

The timolol ophthalmic solution used in this study consisted of  
(0.25%) or 0.5 mg (0.5%) timolol per ml. Benzalkonium chloride was  
added as a preservative. Administered once daily, timolol was dropped  
four times daily, at 9.00 and 21.00 hours.

Diurnal pressure curves were recorded with patients receiving  
miotics (I) and when no glaucoma therapy was given (II). These were  
compared with diurnal pressure curves recorded with open angle  
glaucoma at the beginning (III) and at the end (IV) of the study.

Ocular pressures were measured daily with a standard Schiotz tonometer at 10 00 14 00 and 18 00 hours. Each diurnal pressure curve was recorded two to nine days (mean 3.6). Additionally, applanation tonometry was performed twice daily. On the initial visit the patients underwent a careful ocular examination itemized from 1 to 12 in Table I. The examinations listed 1-7 were carried out on the patients when they were untreated and again on timolol. On the final visit examinations 1 to 10 were made. Additionally the patients were seen two, six, and twelve weeks into the follow-up period and examinations one through four in Table I were carried out. If the patient was uncontrolled, timolol was first added to the maximum of 0.5% timolol twice daily and then pilocarpine 2% three to four times daily. The ophthalmoscopic appearance of the optic discs was compared with the stereophotographs. Blood pressure and pulse rate were recorded daily. On each visit the patients were questioned about subjective local and systemic side effects. The statistical significance of the responses to different treatments was evaluated by dependent t statistics.

*Table I*  
Examinations carried out during the trial

Slit lamp examination	
Applanation pressure of the eye	
Blood pressure and pulse rate	
Ophthalmoscopy	
Corneal thickness and anterior chamber depth	Haag Streib Pachymeter I II
Outflow facility	Electronic Schiotz tonographer Berkeley
Pupillary diameter	Goldmann perimeter
Central visual field	Friedmann analyser
Peripheral visual field	Goldmann perimeter
Schirmer test	
Gonioscopy	
Stereophotography of the optic disc	Allen stereo separator mounted on a Zeiss fundus camera



## Results

The mean intraocular pressures of the diurnal pressure curves (MIOP) of the trial are presented in Fig. 1. In simple glaucoma (MIOP (I) and (II)) in all eyes, however, in two patients (3 eyes) the reduction was not achieved at the beginning of the trial but with additional pilocarpine therapy the MIOP was maintained at a therapeutic level throughout the study. In simple glaucoma MIOP (III) at the beginning of the follow up was 9.6 mmHg or 31% lower than MIOP (II) without therapy ( $P < 0.001$ ) and 3.6 mmHg or 11% lower than MIOP (I) with miotics ( $P < 0.001$ ). During the follow up MIOP decreased in 12 eyes and the mean change was 1.9 mmHg ( $P = 0.01$ ). The final MIOP (IV) was 7.7 mmHg or 27% below MIOP (II) without therapy ( $P < 0.001$ ) and significantly lower than MIOP (I) with miotics ( $P = 0.05$ ). In capsular glaucoma patient (2 eyes) the MIOP was considered too high as a control (od 2% or 23.75) and after the trial pilocarpine was added to the therapy with subsequent decrease of MIOP to a therapeutic level (15.1 or 12.5 respectively).

In capsular glaucoma MIOP (III) at the beginning of the follow up was 10.5 mmHg or 36% lower than MIOP (II) without therapy ( $P < 0.001$ ) and 6.3 mmHg or 25% below the miotic MIOP (I) ( $P = 0.001$ ). During the follow up an increase of IOP was recorded in three eyes and pilocarpine was added to the therapy. The final MIOP (IV) at six months was 7.5 mmHg or

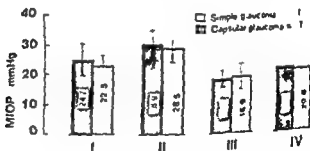


Fig. 1

Mean intraocular pressure of diurnal pressure curve (MIOP) in simple and capsular glaucoma.

MIOP with miotics (I) and without glaucoma therapy (II) compared with MIOP at the beginning of the follow up with optimal timolol medication (III) and at the end of the six month follow up (IV). In simple glaucoma during the follow up MIOP decreased in 12 eyes and the mean change was 1.9 mmHg. In capsular glaucoma at the end of the follow up three eyes were treated together with pilocarpine.

Vertical lines indicate standard deviation.

# Timolol in Simple and Capsular Glaucoma

Table II

ity of outflow ( $\mu\text{l/mmHg/min}$ ) in simple and capsular glaucoma In simple  
oma facility of outflow improved in 13 eyes out of 17 and showed no change  
in capsular glaucoma

		I	II	III	IV
Simple glaucoma	mean	0.126	0.110	0.142 )	0.140 )
	SD	0.041	0.036	0.061	0.074
I P = 0.1		II vs III P = 0.01		II vs IV P = 0.005	
IV P = 0.9					
Capsular glaucoma	mean	0.051	0.060	0.05	0.051*)
	SD	0.020	0.026	0.031	0.035
I P = 0.03		II vs III P = 0.09		II vs IV P = 0.2	

n three eyes miotics were co administered  
or key to Roman numerals see legend for Fig 1

low MIOP (II) without therapy ( $P = 0.02$ ) and 3.3 mmHg or 13.4% lower  
MIOP (I) with miotics ( $P = 0.2$ ) The final recordings showed an un  
trolled IOP of another three capsular glaucoma eyes With additional  
carpine also to these eyes the MIOP was 5.5 mmHg or 22% below MIOP  
with miotics ( $P = 0.04$ )

The values of outflow facility (Table II) were below normal throughout the  
ly in all eyes In simple glaucoma timolol induced a statistically signi  
cant increase of outflow facility At six months the effect was still present  
was not different from the outflow facility with miotics In capsular glau  
ma timolol unlike pilocarpine produced a nonsignificant increase of outflow  
ility

At the beginning of the study after miotics were stopped the pupillary dia  
ters were normalized and they were not affected by the subsequent timolol  
rapy The pulse rate (Fig 2 top) and the mean arterial blood pressure  
g 9 bottom) were initially lowered At polyclinic controls however the  
adings were no longer significantly different from initial values At the six  
nths control the pulse rate was again lowered after three days on the ward  
t the mean arterial blood pressure showed no significant change  
The Schirmer test was made at the first and at the last visit. The tear flow

aqueous humor. This is in agreement with earlier studies (Zetterstrom 1971, Kriegelstein 1978, Sonntag et al. 1979).

The pulse rate was lowered at the beginning and at the end of the study when the patients were examined as inpatients for a few days. This may not be due to systemic absorption of timolol since the average dose was small (ca 0.5 mg daily at the most) and because no significant decrease in pulse rate was measured when the patients were examined as outpatients. A more likely cause is the few days' rest in the clinic and the loss of excitement and anxiety often experienced by patients while at hospital. A reduction of systemic blood pressure was detected with intraocular pressure diminished perfusion pressure of the optic disc.

Some  $\beta$ -adrenergic blocking agents have local side effects because of their local anaesthetic properties (Kriegelstein et al. 1974), metoprolol caused allergic reactions (Ros et al. 1974), oxprenolol produced a bradycardia (Holt & Waddington 1975). Systemic practolol induced oculomotoric syndrome (Wright 1975) the pathogenesis of which is possibly of autonomic origin (Rahi et al. 1976). Mackie et al. (1974) reported that the tear film concentration of practolol in patients was very low suggesting an impairment of the lacrimal gland. They tested also patients receiving oral timolol and the values were found either normal or somewhat increased. In this study no allergic reactions or decrease of the tear flow was seen, corneal thickness remained unchanged and no subjective or objective signs of dry eyes were observed.

Some of the patients in this study expressed a need for a refractive correction. This as well as the increase of the anterior chamber depth may be due in part to a gradual loss of the pilocarpine induced accommodation myopia.

The AC/D, however, increased significantly also during the follow-up in eyes which were treated only with timolol (van Alphen (1971) report). The human ciliary muscle carries mainly  $\beta$  receptors and that the release of catecholamines in eserine precontracted ciliary muscle strips can be blocked by propranolol. Therefore the reason for AC/D increase during treatment is obvious and needs further investigation.

### Acknowledgment

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## ANT-EGG CATARACT

### An Electron Microscopic Study

BY

HENRIK DAA SCHRÖDER and STEEN HOLST NISSEN

The ultrastructure of the ant egg cataractous lens has been studied. Comparison of tissue demineralized by means of EDTA with untreated tissue showed the calcium salts in the ant eggs to be mostly crystalline.

A laminar appearance of the ant egg seen in EDTA treated material suggested an intermittent growth of the structure.

In the ant eggs as well as in some areas separate from these membrane limited cytoplasmic bodies could be seen in many cases the membranes of which were partly joint and partly separated by an electron dense material.

It is suggested that the calcifications seen as the ant eggs are secondary phenomena to an abnormal metabolism morphologically seen as membrane limited cytoplasmic bodies.

**Key words:** ant egg cataract – ultrastructure – calcification – metabolic disorder

The ant egg cataract is a hereditary zonular cataract characterized by a greyish opaque zone containing numerous ant egg like bodies (Ruse 1967, Nissen & Schröder 1978). A recently published paper has dealt with the clinical findings and the heredity of this type of cataract as well as the light microscopic appearance and the element content of the ant eggs (Nissen & Schröder 1978).

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Figs. 1 and 2

1 Transition zone between an ant egg and the surrounding tissue. In the left part of the micrograph many crystals (C) are to be seen as very dark lines. Also less calcified tissue membrane limited cytoplasmic bodies with (M) and membrane appendices are found.  $\times 70,000$

2 Transition zone after EDTA treatment. The crystal seen in Fig. 1 has disappeared but empty spaces (examples at E) with a localization and shape as in Fig. 1 are seen instead. The dense material (D) and the membranes (M) have been treated.  $\times 70,000$

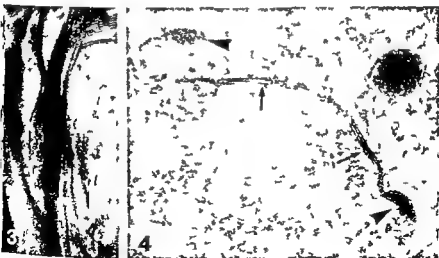
The present study was undertaken in order to further elucidate the structure of the ant egg. In addition a structural explanation of the opaqueness of the lens of the ant containing the ant eggs was looked for.

### Material and Methods

Anterior tissue from an ant egg cataract was fixed in cold 3% glutaraldehyde buffered with 0.15 M phosphate buffer (pH 7.0). After 3 h of fixation the tissue was fixed for one h in 1%  $\text{OsO}_4$ , dehydrated in graded ethanol and embedded in Araldite 81. Crude aspirate as well as isolated ant eggs were embedded in Araldite 81. Part of the material was demineralized for 5 days in 10% EDTA in 0.1 M Tris buffer pH 7.3 at 4°C before postfixation and embedding (Fullmer & Link 1964). Ultrathin sections were cut and stained with uranyl acetate 30 min and lead citrate 10 min.

### Results

Electron microscopically the ant egg has been described as consisting of a homogeneous core surrounded by a zone of a wavy appearance at the transition to apparently normal tissue (Nissen & Schroder 1978).



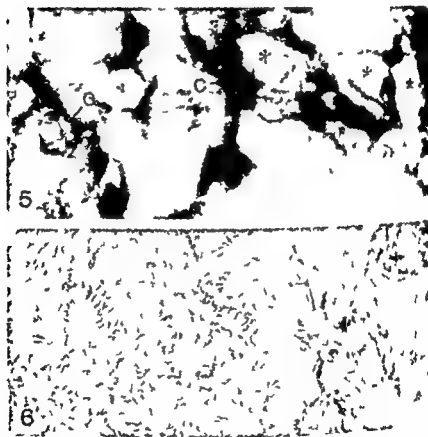
*Figs 3 and 4*

Crystals seen in the transition zone. These laminated structures disappear when subjected to EDTA treatment  $\times 100\,000$ .

High magnification of a membrane limited cytoplasmic body. The five layers of the joint membranes (arrows) as well as the swellings containing dense material (arrowheads) are seen  $\times 100\,000$ .



The transition zone was at electron microscopic level characterized by interdigitation of irregular relatively electron dense processes (Fig 1) and more translucent processes from the surroundings (Fig 1). Between these two components a distinct dark layer of variable thickness was often found. At high magnifications this was seen to be lamellar. Structures of a similar appearance could also be seen in the distal part of the transition zone. In the EDTA treated material these lamellae had disappeared leaving empty spaces (Fig 2).



Figs 5 and 6

5: Ant egg core. The hardness of the calcified core results in a partial loss of the tissue during the sectioning with a consequent loss of residual lamellae (C) are however seen in the remaining tissue. 5000x

6: Core of the ant egg after treatment with EDTA. The crystals are dissolved leaving empty needle shaped spaces smaller and more regular to the left than to the right. 21000x

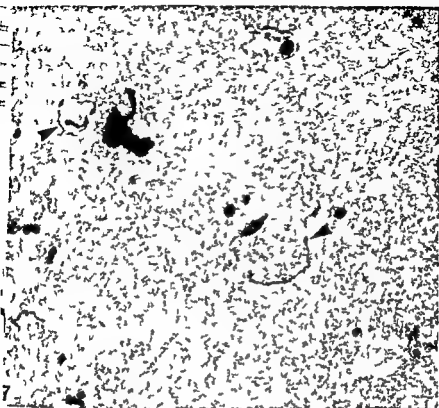


Fig. 1

Electron micrograph from a tissue block not containing ant eggs. Several cytoplasmic bodies with associated membrane material are seen (examples at arrowheads)  $\times 90\,000$

In the periphery of the transition zone electron dense bodies surrounded by an approximately 11 nm thick membrane were found (Fig. 1). These bodies were often continuous with some linear structures which at higher magnifications appeared to be five layered with alternating light and dense lines (Fig. 4). The central dense line was often more electron dense and thicker than the two peripheral dense lines. The five layered structure most likely represented a fusion of two adjacent membranes.

The membrane limited cytoplasmic bodies resisted EDTA treatment (Fig. 2). The core of the ant egg was not easily studied in mineral containing tissue and only a few laminated structures were seen (Fig. 5). When decalcified a picture very similar to that of the border zone was observed apart from a more needle shaped appearance of the dissolved structures in the core (Fig. 6). It

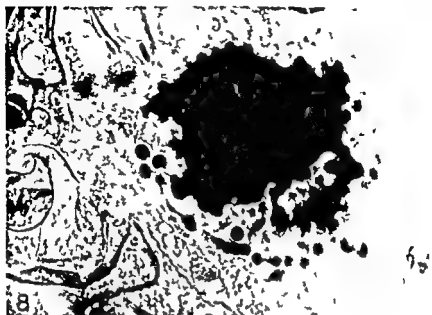


Fig. 9

Membrane limited globules and processes most of which are lysed and  
of fibre cytoplasm  $\times 20000$

appeared as if the core could be separated into more concentric shells. The structure differed in respect to size and density of the needle shaped structures. Cytoplasmic bodies similar to those seen in the border zone were present in the core.

In some pieces of tissue not containing ant eggs areas with electron dense cytoplasmic bodies and associated joint membranes were found. Their appearance was similar to that seen in the transition zone of the ant eggs. Their size and shape was more variable (Fig. 10).

In the present material no ribosomes were observed. Some small, electron dense bodies were found and some electron dense material bearing similarities to the material seen in the ant eggs as well as some with a less obvious origin (Fig. 11) was seen.

## Discussion

The appearance of the laminated curved structure of the border zone is similar with that of a crystalline structure. The disappearance of the structure after EDTA treatment indicates a calcium salt nature of the crystalline structure.

ement with the previous recordings of calcium and phosphorus in the ant (Nissen & Schroder 1978). The finding of crystal like structures in the core, the similarity in effect of EDTA on the core and the transition zone of the egg resulting in needle shaped empty spaces indicate that also much of calcium and phosphorus of the core is present as crystals.

The existence of more concentric shells in the core of the ant eggs as well as the presence of a border zone would be in agreement with a progressive but intermittent growth of the ant egg. It has however not been possible to obtain information on the development of the ant egg cataract in the previously published papers.

The localization of the electron dense bodies with appendices in the vicinity of the ant egg as well as in tissue blocks not containing ant eggs suggests that these structures may be the structural background of the opaqueness found outside the ant eggs. However, due to the mixture of lens material during aspiration, it is impossible to prove that the dense body containing material originates from the periphery of the lens.

The fusion of two adjacent membranes has previously been described in the thyroid hormone stimulated osteoclast (Lucht & Maunsbach 1973). Here rER were found to be a part of the endoplasmic reticulum and they were taken as evidence for an increased synthesis of membranes. In the present study however the deficiency of ribosomes means that no significant protein synthesis is taking place. It is however a possibility that the complexes are part of the smooth endoplasmic reticulum. This organelle is known to be involved in the synthesis of lipids and polysaccharides which could account for the presence of electron dense material within the structures.

Another possible interpretation is that the cytoplasmic bodies could be more or less collapsed lysosomes. They could then be involved in an autophagocytosis. Also the possibility that the cytoplasmic bodies are secretion vacuoles has been mentioned.

None of the mentioned organelles are seen in normal lens fibres (Cohen & Rafferty & Goossens 1977). The presence of the cytoplasmic bodies therefore indicates that the normal metabolism of the lens fibres is disturbed. In other studies on lens fibre ultrastructure in cataracts an increased amount of membrane material appeared (Dilley et al 1976) and dense bodies or large vacuoles with an appearance similar to that seen in the present Fig. 8 were also observed. However, in none of these studies could membrane limited cytoplasmic bodies similar to those characteristic for the ant egg cataract be found. In other types of cataracts including congenital cataracts an altered metabolism may lead to necrosis and precipitation of various compounds such as calcium salts (Duke Elder 1969). Thus the calcium containing ant eggs which

clinically characterize the ant egg cataract might well be the presence of but unspecific calcium deposition due to an altered metabolism of the lens fibres morphologically observed as cytoplasmic dense bodies.

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## ANTERIOR CHAMBER HAEMORRHAGE IN THE NEWBORN AFTER SPONTANEOUS DELIVERY

### A Case Report

BY

PEKKA POHJANPELTO KYRÖ NIEMI and TIMO SARMELA

A case report is presented of anterior chamber haemorrhage occurring in one eye in a newborn after spontaneous delivery. At the age of two weeks the anterior chamber was clear but the vitreous cloudy. At the age of five weeks the vitreous had also cleared. The infant's later development was normal and there were no disorders in the function of the eye.

**Key words:** birth injury - intraocular haemorrhage in the newborn - hyphaema

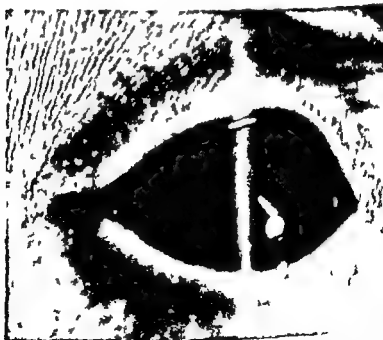
Anterior chamber haemorrhages in the newborn are common. The reported incidences vary from 26 to 50 per cent (Chace et al 1950, Kauffman 1958, Krauer 1965, Neuweiler & Onwudiwe 1966). The big variation seems to be due to case selection and time of examination. Owing to the rapid absorption of retinal haemorrhages are most commonly noted shortly after birth. Haemorrhages into the vitreous are considerably more infrequent. Wiza & Wiza (1976) collected in addition to their own two patients 10 reports describing a total of 18 cases from the earlier literature. Anterior chamber haemorrhage in the newborn is an exceptional birth injury. The rare cases reported in the literature involved forceps delivery. In these cases the haemorrhage into the anterior chamber was the consequence of ocular trauma caused by the forceps leading to the infant's death (Homer

1884 Lomer 1894 Wintersteiner 1899 Thomson & P (1923) et al (1974) reported on a newborn with hyphaemas which were caused by the forceps. The infant was diagnosed to have a coagulation and died 36 h after birth.

This is a report on a case in which anterior chamber haemorrhage was established in a newborn after spontaneous delivery where a mild cloudiness probably also blood was observed in the vitreous body.

The mother was a healthy primipara of 23 years. Proteinuria had been noted in the 32nd week of pregnancy and occasionally an elevated blood pressure was recorded. No treatment was given. Labour began spontaneously in the 38th week of pregnancy. It lasted 4 h and 25 min, the duration of the second stage of labour 15 min. The amniotic fluid was normal. The baby, a girl, was delivered by cephalic presentation. A coil of the umbilical cord emerged during the presenting part. Delivery of the shoulders progressed with difficulty.

The infant's birth weight was 3900 g, length 50 cm and head circumference 34 cm. Her general condition was good apart from mild cyanosis. The Apgar score was 8. There was slight facial haematoma. The left arm was hypoplastic which was due to a fracture of the clavicle.



*Fig. 1*  
Infant's left anterior chamber full of blood at the age of 4 weeks.

## *Anterior Chamber Haemorrhage in the Newborn*

Examination of the infant's eyes at the age of two days showed them to be abnormally red and an ophthalmologist was consulted.  
On the 3rd day of life Both conjunctivas revealed minor haemorrhages. The left anterior chamber was full of blood of coagulated appearance (Fig 1). The right anterior chamber was completely clear macroscopically.  
At two weeks Both anterior chambers were clear on slit lamp examination. The vitreous was clouded and the red reflex absent. The right vitreous was clear.  
At five weeks Both anterior chambers and vitreous bodies were clear.  
Follow up at 3½ years Vision of the right eye was 0.8 refraction +1.5 sph +0.5 cyl. At 30° there was some pigment on the surface of the lens remnants of a pupillary membrane were not observed, the anterior chamber and vitreous were clear. The ophthalmoscopy finding was normal. The vision of the left eye was 0.8 refraction +1.5 sph there were slight remnants of a pupillary membrane and some pigment was visible on the surface of the lens the anterior chamber and vitreous were clear. The child moved freely and the iris appeared normal without any abnormal transparency. Ophthalmoscopy finding was normal. Gonioscopy in the chamber angle open anterior iris processes no recession of the angle no iris synechiae. Gonioscopy of the left eye was not performed because of the co-operation problems with a young child. Pediatric examination disclosed nothing abnormal compared with a healthy child. The girl had been healthy throughout. The mother had not detected anything indicative of an increased bleeding tendency. The blood values were considered to be normal. The thrombocyte count was 400 000 mm<sup>3</sup> bleeding time was 2 min 30 sec and clotting time 150 sec.

## Discussion

To our knowledge not a single case of anterior chamber haemorrhage in the newborn after spontaneous delivery has been reported in the earlier literature. In a large series up to 10 000 patients in which retinal haemorrhages of the newborn were studied contain no mentions of hyphaema which would be very easy to diagnose as a subsidiary finding (Chace et al 1950 Kauffman 1958 Bauer Mayer 1965 Neuweiler & Onwudiwe 1966). Birth traumas in the previously reported cases of anterior chamber haemorrhage in the newborn were so severe that the patients died. The child described here was not found to have any other injuries except for the fracture of the clavicle and her development was normal. The blood had disappeared from the anterior chamber at the age of two weeks and the vitreous had cleared three weeks later. The vitreous opacity was probably also due to haemorrhage although its absorption generally takes several months in a newborn (Braendstrup 1969). Retinal haemorrhages of the newborn have been attributed to congestion of retinal veins during delivery. This aetiology does not seem to be correct regarding anterior chamber haemorrhage. The causative agent is probably a mechanical trauma for hyphaema of the newborn is extremely rare and in a g



the case now reported it was unilateral. The causative agent in the previously described has been a mechanical lesion caused by forceful traction. In exception is possible the patient described by Azar et al (1974) was found to have haemorrhages associated with disseminated intravascular coagulation in different organs. A possible causative agent in this case was the umbilical cord which was forced out simultaneously with the placenta.

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# FIBRINOLYSIS AND TRAUMATIC HYPHAEMA

BY

THORKILD BRAMSEN

Since January 1st 1975 310 patients with traumatic hyphaema have been treated with the antifibrinolytic drug tranexamic acid. One secondary haemorrhage has occurred corresponding to a frequency of secondary haemorrhage of 0.32%. Eighty five of these patients were treated as out patients.

In four patients with traumatic hyphaema who were not treated with tranexamic acid, the serum content of activator inhibitor was determined daily. An increase was seen during the first five days after the trauma followed by a marked fall on the 6th day. In the same four patients the central corneal thickness was followed by daily measurements and compared to the variation in activator inhibitor.

**Key words:** traumatic hyphaema - fibrinolysis - tranexamic acid - activator inhibitor - central corneal thickness

has been previously described (Bramsen 1976, 1977) the antifibrinolytic drug tranexamic acid (Cyklokapron®) has been used in the treatment of traumatic hyphaema on the supposition that secondary haemorrhages occur on a fibrinolytic basis. This supposition is supported by the favourable results obtained in the previous studies (secondary haemorrhage frequency 0.68%) and the time of onset of the secondary haemorrhages also indicates a fibrinolytic origin. Moreover Pandolfi (1978) has stated that high concentrations of fibrin degradation products (FDP) are found in the aqueous humour of patients with secondary haemorrhages. This demonstrates that fibrinolysis occurs in the anterior chamber in these patients. Pandolfi & Kwaan (1967) also found that the tissue and to a lesser extent cornea endothelium was rich in plasminogen activators. For ethical reasons it is not possible to study the fibrinolytic

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activity in the aqueous humor in humans at different times after a traumatic hyphaema. However, the possibility that trauma may affect the fibrinolytic system of the whole organism has not been examined. Ygge (1972) studied the changes in concentration in the serum of fibrinolytic factors among others the factor activator and inhibitor after abdominal operations. He found that the activity of this factor was markedly increased during the first 5-6 days only to fall back around the 10th to 7th day. It is understandable that abdominal operations influence the fibrinolytic activity of the whole organism and it is of interest to examine whether trauma to the eye is likewise accompanied by changes in the serum concentration of activator and inhibitor.

Knight et al (1977) showed that changes in fibrin lysis in the aqueous humor depended upon the individual response which differed from patient to patient and did not depend upon the extent of the surgical trauma. As it was not possible to examine the changes in the content of the aqueous humor in these patients it was instead decided to examine the changes in the serum by taking daily blood samples. It was essential that the patients who took part in this study did not receive any form of antifibrinolytic treatment.

In a double blind trial by Bramsen et al (1978) it was shown that tranexamic acid had a reducing effect on cornea oedema and a possible connection between the fibrinolytic system and central corneal thickness was demonstrated. In those patients where the serum content of activator and inhibitor was determined measurements of central corneal thickness were also taken in order to determine whether there was any connection between the changes in these two parameters.

Tranexamic acid is a potent antifibrinolytic drug (Bramsen et al 1977) during bodily exercise (Biggs et al 1947) but can be effectively used to prevent bleeding from the assumption that secondary haemorrhage occurs on a fibrinolytic basis it should therefore be unnecessary to administer any form of antifibrinolytic drug in patients with traumatic hyphaema when treated with an antifibrinolytic agent.

In a study by Bramsen (1977) 70 patients with traumatic hyphaema were treated with tranexamic acid. The patients were mobile but no case of secondary haemorrhage occurred. In the present study the results in patients treated on an out patient basis are presented.

## Material

The examination took place between 1.1.1978 and 31.12.1978. From the material comprised 4 patients referred to the department with traumatic hyphaema. Only those patients with macroscopic hyphaema were included in the study.

## *Traumatic hyphaema*

78 patients consisted of 64 males (average age 17.5 years with a range from 5-ears) and 14 females (average age 23.2 years with a range from 7 to 45 years) other eye lesions apart from hyphaema are shown in Table I

From 1/1-1/10 1978 the material comprised 85 patients with traumatic hyphaema during this period only those patients with macroscopically visible hyphaema included. The patients consisted of 63 males (average age 24.1 years with a range from 5 to 71 years) and 22 females (average age 23.6 years with a range from 7 to 77 years). The eye lesions in addition to the hyphaema are shown in Table I

Our male patients with macroscopically visible hyphaema took part in the daily determinations of activator-inhibitor content in the serum and measurements of central corneal thickness. The ages of these patients were 27, 22, 24 and 54 years respectively. Other eye lesions apart from hyphaema are shown in Table I

## Methods

Patients with the exception of the four who had the serum content of activator-inhibitor measured were treated with oral tranexamic acid 25 mg/kg/day three times daily. This was the sole form of treatment and started on the day of referral. From the period 1/1 1977 until 31/12 1977 the patients were admitted to the ward for a period of five days but they were mobile without any eye bandaging. There were no restrictions in the patients' physical activity. From the period 1/1 1978 until 1/10 1978 patients were admitted to the ward on the day of referral and instructed in the

*Table I*

	Period 1/1 77 until 31/12 77	Period 1/1 78 until 1/10 78	Four patients treated with bed rest
Subconjunctival haematoma	8	10	1
Corneal erosion	96	24	2
Pupillary changes	46	41	3
Iridodialysis	3	4	
Increased intraocular tension	■	10	1
Traumatic cataract	9	1	
Retinal haemorrhage	4	7	
Central retinal oedema	■	8	
Choroidal rupture	3	■	
No associated lesions	12	14	-
No	78	85	4

Lesions associated with traumatic hyphaema

proper administration of tablets. The patients from this period were from the following day and allowed to resume their normal work and activities. On the day following the trauma all the patients were re-examined including a visual examination, ophthalmoscopy, tonometry and visual acuity. Complications were formed in patients over 10 years of age. Twelve patients did not show any examination.

The four patients who did not receive the antifibrinolytic treatment at the time of admission, treated with bed rest and stenopaeic glasses. This treatment continued for six days. At the same time daily measurements of central corneal thickness were performed, and venepuncture was carried out to determine the inhibitor content. The estimation of this factor was carried out in the laboratory. The venous blood was kept in a plastic container at  $-60^{\circ}\text{C}$ . The analysis of the inhibitor was carried out using the method described by Parakevas et al. (1977). A pool of venous blood from 40 healthy individuals was used as a standard for the examination. A standard curve was drawn from a dilution series of this pool (1:1, 1:2, 1:10, 1:20 and 1:40). The percentage concentration of the inhibitor content in the patients' serum was determined from this curve. The principle of the method is as follows - fibrinogen, thrombin, plasminogen and urokinase are added to the patients' serum. The fibrinogen is changed by the thrombin into a fibrin clot. The urokinase activates plasminogen into plasmin. The plasmin dissolves the fibrin which then becomes no longer visible. The time interval until the clot is dissolved depends upon the serum content of inhibitor.

## Results

No secondary haemorrhages occurred among the 78 patients who were admitted to the ward without being confined to bed during the period 1/12/77 until 31/12/77. Examination on the 12th day revealed visual acuity and gonioscopic findings as shown in Table II. Neither were there any secondary haemorrhages among the 80 patients treated as outpatients in the period 1/1/78 until 1/10/78 in spite of the fact that almost all the patients resumed their normal working activity. The visual acuities and gonioscopic findings on the 12th days are shown in Table III.

In the four patients treated with bed rest, without antifibrinolytic treatment there were no secondary haemorrhages. Fig. 1 shows the average curve of the variations in the serum content of activator-inhibitor ( $\bar{x} \pm \text{SD}$ ) and the average curve of the variations in the central corneal thickness ( $\bar{x} \pm \text{SD}$ ). As can be seen from the curve of the activator-inhibitor content, a decrease occurred during the first two days (concentration about 50% of normal). Following this the inhibitor in the serum increased and reached a maximum on the 5th day (165%). Between the 5th and 6th day a fall occurred from 165% to around the normal of 100%.

# Traumatic hyphaema

Table II

Visual acuity		Gonioscopy	
1 0	46	Normal	36
0.5-0.9	18	Goniosynechias	4
0.3-0.4	3	Traumatic angle	10
0.1-0.3	2		No
0.1	1		No

The visual acuities and gonioscopic changes on the 12th day following traumatic hyphaema in 8 in patients treated with tranexamic acid for 6 days following the trauma Eight patients did not show up for re examination

The average curve of the central corneal thickness showed an unchanged demo for the first two days This was followed by a fall during the period tween the 2th and 5th day (during which time the inhibitor in the serum creased) A secondary increase in thickness occurred between the 5th and h day to be followed by a renewed fall in thickness The secondary increase i thickness occurred simultaneously with the marked fall in serum content of tivator inhibitor

Table III

Visual acuity		Gonioscopy	
1 0	62	Normal	52
0.5-0.9	13	Goniosynechias	8
0.3-0.4	3	Traumatic angle	3
0.1-0.3	3		
0.1	0		

The visual acuities and gonioscopic changes on the 12th day following traumatic hyphaema in 80 out patients treated with tranexamic acid for 11 days following the trauma Four patients did not show up for re examination

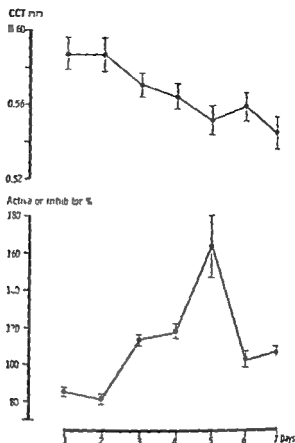


Fig 1

The average curve of the variations in the serum content of activator  $\pm$  SEM, and the average curve of the variations in the central corneal thickness ( $\pm$  SEM, mm) in patients with traumatic hyphaema

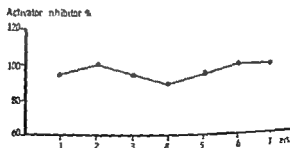


Fig 2

The variation in the serum content of activator inhibitor in a healthy member of staff in the department

## Discussion

Antifibrinolytic treatment is considered to be effective in preventing secondary haemorrhage following traumatic hyphaema. In the course of 1975, 12 patients with traumatic hyphaema were treated with bed rest, stenopaeic glasses and tranexamic acid, and one case of secondary haemorrhage occurred (Bramsen 1976). During 1976, 75 mobilised patients were treated with tranexamic acid, and no secondary haemorrhages occurred (Bramsen 1976). This paper presents the results of the antifibrinolytic treatment of 78 mobilised inpatients and 10 outpatients. There were no cases of secondary haemorrhages. Altogether 10 patients with traumatic hyphaema have been treated with tranexamic acid, with one case of secondary haemorrhage corresponding to 0.32%. Of these only 12 patients have, together with the antifibrinolytic treatment, been treated with bed rest (the one case of secondary haemorrhage occurred in this group). The present material therefore suggests that restriction in physical activity during antifibrinolytic activity is not relevant as far as the occurrence of secondary haemorrhage is concerned.

Studies on the changes in the central corneal thickness and the serum content of activator-inhibitor were only undertaken in four patients. The results suggest that changes in both parameters occur following ocular trauma. In the present study, there was a close correlation between the changes in the central corneal thickness and the serum content of activator-inhibitor, in that the corneal thickness decreased while the inhibitor in the serum increased. A secondary increase in corneal thickness occurred at the time of a fall in the concentration of activator-inhibitor. Bramsen & Stenbjerg (1979) studied the same factor in the serum after operative intervention on the eye and found a similar correlation to the changes in corneal thickness in the majority of the patients.

As described in the introduction, various authors have studied the changes in the fibrinolytic activity in connection with operative interventions, and similar results have been found. As mentioned, Knight et al. (1977) found that these changes were independent of the extent of the operative trauma. These authors examined the fibrinolytic response to trauma in 53 patients and found that a fall in fibrinolytic activity from the day following operation occurred in 81% of patients, whereas 19% had a higher activity on the first postoperative day than preoperatively, and here the fall in activity during the following days was gradual and slight. Secondary haemorrhages could possibly occur in this last group.

In the Nordic countries, the frequency of secondary haemorrhages lies considerably below 19%. This could be due to the fact that the treatment



with bed rest is effective. At the present time it is not possible to prevent patients who are capable of effectively inhibiting fibrinolysis after a trauma. As secondary haemorrhages have such serious consequences it must be possible to treat all such patients with an antifibrinolytic agent.

The repeated venepunctures on the patients in whom measurement of plasminogen activator inhibitor were undertaken can be said in themselves to be traumatic. Daily blood samples for measurement of serum activator inhibitor concentration were taken from one of the members of the staff in the department in order to determine whether the taking of blood samples could cause changes. The result is shown in Fig. 2. The changes are quite minimal.

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# SERUM AND AQUEOUS HUMOUR CONCENTRATION OF TRANEXAMIC ACID AFTER PERORAL ADMINISTRATION

BY

THORKILD BRAMSEN

After a single oral dose of 20 mg/kg tranexamic acid in 37 patients with cataract as the sole eye disease the concentration of the drug was measured in both the serum and the aqueous at various intervals following intake

The serum concentration was highest after three h (average 10.44 mg/l) but a trace of tranexamic acid (0.7 mg/l) could still be found after 19 h. In the aqueous the concentration was likewise highest after three h (average 1.67 mg/l) but following this the fall in tranexamic acid concentration was very gradual and after 11 h it was found to be 1.3 mg/l.

In two patients who had received 20 mg/kg three times daily for three days an aqueous concentration of 0.3 mg/l was found eight h after the final intake.

**Key words:** Aqueous - cataract - tranexamic acid - serum

In ophthalmology tranexamic acid has been used in the treatment of hyphaema (Bramsen 1976, 1977) and in the prevention of secondary haemorrhages following cataract operations (Jerndal & Frisen 1976). The drug has also been shown to be able to reduce corneal oedema in Fuchs' endothelial dystrophy (Bramsen & Ehlers 1977) and to reduce the corneal oedema occurring after cataract operations (Bramsen et al 1978). Moreover tranexamic acid has been used in the treatment of choroidal melanomas (Bramsen 1978).

Previously there has only been indirect evidence in favour of the drug passing the blood aqueous barrier. The effect of the drug on pathological condi-

tions in the anterior segment could be indirect e.g. via the fibrinolysis, the complement system or the amino acid transport system. As the aqueous concentration after intake of tranexamic acid is therefore of importance for the understanding of the mechanism of action of the drug. There is no report in the literature of any studies in humans concerning this. Campbell (1976) showed that tranexamic acid was able to penetrate the cornea but apart from this the tranexamic acid concentration in the aqueous has not been studied.

Davson (1956) has pointed out the similarities between the composition of cerebrospinal fluid and aqueous humor. Tovi & Thulin (1972) reported on the concentration of tranexamic acid in the serum and cerebrospinal fluid following injections of 1000 mg every hour for eight days. The examination was carried out on four patients who were treated with tranexamic acid in order to prevent secondary haemorrhages after aneurysmal subarachnoid haemorrhages. The examination showed that the concentration in the cerebrospinal fluid gradually increased following repeated injections, whereas serum concentration was not affected by this. A concentration of 0.3 mg/l was found in the cerebrospinal fluid 30 minutes after injection. The concentration after 8 days treatment was 4.5 mg/l. At the same time measurements of the cerebrospinal fluid concentration of fibrin split products (FDP) which is a sign of the breakdown of fibrin were also undertaken. The concentration of FDP had fallen after 30 min in spite of the low concentration of tranexamic acid at that time. FDP was no longer found in the cerebrospinal fluid after eight days.

Tovi & Thulin (1972) have shown that tranexamic acid is capable of passing from blood to cerebrospinal fluid. It is however to be noted that the patients in the study had suffered subarachnoid haemorrhages and consequently the blood-brain barrier was most probably no longer intact. This could explain the increase in concentration. In the following study the results of the investigations of tranexamic acid content in the aqueous of the eye are reported. It is considered that the blood-aqueous barrier is intact.

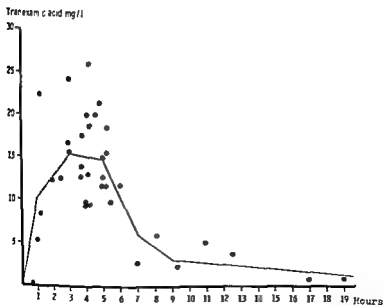
## Material

The material comprised 39 patients who had been admitted for senile cataract operations. The average age was 67.5 years with a range from 50 to 83 years. On admission a routine ophthalmological examination was performed to exclude the presence of additional eye disease. Only those patients where senile cataract was the only disease were accepted for the study. Serum creatinine was determined in all patients and only those with normal values were accepted.

## Methods

Seventy-seven patients received oral tranexamic acid 25 mg/kg at various time intervals before operation (from 45 min to 19 h). The cataract operation commenced under bulbar anaesthesia with paracentesis of the anterior chamber and aspiration of 200 µl of aqueous. In two patients the amount of aqueous aspirated was insufficient for determination of the tranexamic acid content. Venepuncture for determination of the serum content of tranexamic acid was performed as soon as possible before or after the anterior chamber paracentesis. The aqueous and serum were immediately frozen to -18°C and in the frozen state were sent to the research department in Stockholm where gas chromatography analysis was performed as described by Sjoman & Stromberg (1975). Two patients were treated with oral tranexamic acid 25 mg/kg three times daily for three days and paracentesis and venepuncture were performed eight h after the last dose.

The  $S_{10}$  values for serum concentration and the  $S_{20}$  for aqueous concentration were drawn up on a co-ordinate system. Owing to the quite considerable range an average curve was determined in the following way. The average of all the values between 1-2 h were grouped as for one hour. The average of the values between 2-4 h were taken as corresponding to 3 h and so on up to and including 10 h for the serum values and 6 h for the aqueous values.



*Fig. 1*

The serum concentrations of tranexamic acid after a single oral dose of 25 mg/kg measured at various intervals after intake. One point per person.

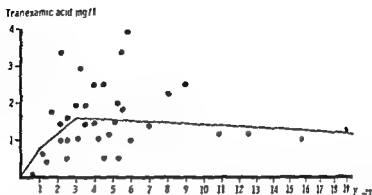


Fig 9

The aqueous humour concentrations of tranexamic acid after a single oral dose of 25 mg/kg measured at various intervals after intake. One point per patient.

## Results

Fig 1 shows the serum concentrations of tranexamic acid after a single oral dose of 25 mg/kg measured at various intervals after intake. It can be seen that the average maximum concentration (15.44 mg/l) is reached after 3 hours. Following this there is a sharp fall between 3-9 h. Tranexamic acid is, however, still found in the serum after 19 h.

Fig 2 shows the aqueous concentrations of tranexamic acid following a single oral dose of 25 mg/kg measured at various intervals after intake. The peak concentration (16.2 mg/l) is also found after about three h. The graph, however, remains almost constant after this, so that even after 19 h, the concentration approximates the 3-h value.

The graphs also show that the aqueous concentration is about 10% of the serum concentration for approximately the first five h. After 1 h the aqueous concentration is higher than the serum concentration.

In the two patients who had received tranexamic acid 25 mg/kg three times daily for three days the serum concentrations were 3.6 mg/l and 8.4 mg/l respectively (average value 6.0 mg/l) eight h after the final intake. This is about 30% higher than the average value found after a single oral dose. The corresponding values for aqueous after eight h were 2.9 mg/l and 2.5 mg/l, which is about 40% higher than the average value after a single oral dose.

## Discussion

present study has shown that tranexamic acid capable penetrating the aqueous barrier and that the concentration during the first five h after single oral dose is approximately 10% of the serum concentration. Tranexamic acid is slowly eliminated from the anterior chamber of the eye. Moreover it is possible that as with cerebrospinal fluid accumulation occurs after repeated intake of the drug. In those cases where the blood aqueous barrier is destroyed e.g. following ocular trauma and with inflammatory conditions in the anterior chamber it can be assumed that a larger concentration than normal can pass into the anterior chamber.

Ørskov & Thulin (1972) found that a concentration of 0.3 mg/l was able to reduce the content of FDP in the cerebrospinal fluid. This suggests that even a low concentration has an inhibitory effect on the breakdown of fibrin. In a healthy eye with an intact blood aqueous barrier it can be assumed that after repeated intake with approximately six h interval the concentration will be higher than 0.3 mg/l. It can therefore be assumed that the concentration is sufficient to inhibit fibrinolysis.

In the case of traumatic hyphaema it is hardly the concentration of tranexamic acid in the aqueous that determines the effectivity of the drug. The primary clot is situated in the vessel wall and the activators of the fibrinolytic system are largely localised in the endothelium of the blood vessels. It is therefore to be expected that it is the serum concentration that determines the effect of the drug.

Moreover the study suggests that there are quite considerable individual differences in the tranexamic acid serum concentration but less in the aqueous concentration after oral intake. In a study on 12 healthy volunteer patients by Eriksson et al. (in press) the serum tranexamic acid concentration was measured at 0, 3, 4, 5, 8 and 9 h after oral and intravenous intake. In this material especially during the first four h a considerable individual difference in the concentration both after oral and intravenous administration was also noted. The pattern of the graph in the present study is similar to that found by Eriksson et al. (1979). The range could be due to the uncertainty in determining the tranexamic acid concentration. The amount of aqueous or serum necessary for the measurement is about 150  $\mu$ l (Veerman & Strömberg 1975). Analysis of samples in biophysiological studies have shown a variation coefficient of 1.4% ( $n=8$ ). Samples with a very low concentration (about 235 mg/ml) had a variation coefficient of 7.4% ( $n=8$ ).

In the present study there is nothing inconsistent with a passive diffusion of tranexamic acid across the blood aqueous barrier. The higher concentration in



## Results

## Newborn human lens

Anterior epithelial cells of the newborn human lens revealed intermediate filaments as well as actin filaments (Fig 1). The actin filaments (5 nm diameter) were mainly seen at the basal region of the epithelial cell close to epithelial fiber cell junction intermingled with 10 nm filaments. Cortical nuclear fiber cells contained intermediate filaments and thin irregular filaments.

Actin filaments were 5 nm in diameter with attached particles (Fig 3). Negative stain analysis has previously shown that the irregular fibrils referred to as chains consist of particles (15–20 nm diameter) arranged along a filament backbone of 5 nm diameter (Bradley & Maisel 1978).

Cortical nuclear fiber cells contained intermediate filaments however the chains were found both in single elongate forms as well as in aggregated configurations (Fig 9).

## 24-year-old human lens

Anterior epithelial cells of the 24 year old lens contained 10 nm filaments and actin filaments as observed in the newborn lens (Fig 4). Incubation of the anterior epithelium with HMM exhibited arrowhead complexes on the actin filament network but not on the 10 nm filaments (Fig 5). Intermediate filaments (10 nm) were present in the superficial cortical fiber cells (Fig 6) markedly increased in number in the deep cortical cells and absent from the nuclear fiber cells. Single elongated chains were abundant in the superficial cortical fiber cells. They appeared in aggregated and coiled form in the deeper cortical and nuclear fiber cells (Fig 7).

## 80-year-old human lens

In the 80 year old human anterior epithelium also contained many 10 nm filaments as well as actin filaments. No other area of the 80 year old lens demonstrated the presence of 10 nm filaments. The protein chains in the cortex and nucleus were seen mostly in the aggregated form (Fig 8).

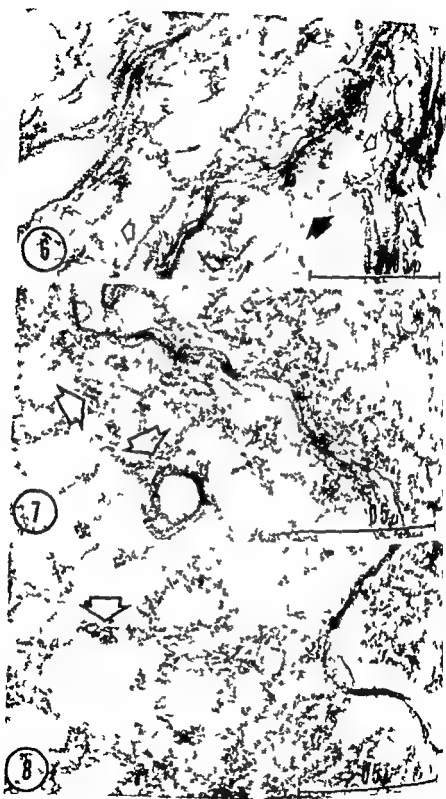
Fig 4

Anterior epithelial cells of 24 year old lens showing 10 nm filaments (solid arrowheads) and actin filaments (open arrowheads). The filaments were present on the epithelial side of the epithelial cell fiber cell junction. 120 000  $\times$ .

Fig 5

Incubation of the epithelial cells with HMM demonstrates arrowhead complexes on actin filaments (open arrowhead) but no reaction on the 10 nm filaments (solid arrowheads). 120 000  $\times$ .





## Discussion

presence of cytoplasmic filaments in lens epithelial cells has previously documented. Actin has been identified by Longchamp et al (1976) in the epithelial cells using immunofluorescence by Mousa & Trevithick (1977) in mouse epithelial cells and by Rafferty & Goossens (1978) in mouse, frog, grey tree squirrel and human lens epithelial cells. Although microtubules were not seen in this study they have been reported in lens epithelial and superficial cortical fiber cells (Kuwabara 1975; Piatigorsky 1975; Bradley et al press). Microtubules are extremely sensitive to low temperature, pH and ionic environment. Under the conditions of this experiment microtubules are easily disrupted.

In their study of the unglycerinated human lens, Rafferty & Goossens (1978) noted a heavy accumulation of filaments on either side of the epithelial fiber cell junction, especially on the cytoplasmic side of lens fibers abutting the overlying epithelium. In this study a heavy concentration of filaments (both actin and intermediate filaments) was noted on the epithelial side of the junction. The absence of a fibrillar sheath on the fiber side of the junction in this study may be due to the experimental conditions.

Although intermediate filaments were present in the epithelial cells in all the ages, their distribution in the fiber cells varied with age. Thus they were found in cortical and nuclear cells of the newborn lens, only in the cortical fiber cell of the 24 year old lens and only in the epithelial cells in the 80 year old lens. Even in the 24 year old lens, the number of intermediate filaments increased markedly from the superficial to deep cortical fiber cells. The absence of intermediate filaments from adult lens nuclear fiber cells is consistent with previous studies on rat, rabbit and chicken lens (Maisel 1977).

The protein chains, previously identified by negative stain as globules arranged along a filamentous background, appear in single elongated form in the superficial cortical cells. In the deep cortical and nuclear cells they appear

Fig 6

Unfixed, unglycerinated superficial cortical fiber cells of the 24 year old human lens contain 10 nm filaments (solid arrowhead) and single elongate forms of chains of proteins (open arrowhead) 84 000  $\times$ .

Fig 7

The nuclear region of the 24 year old lens contains only chains of protein in aggregated forms (open arrowhead) 84 000  $\times$ .

Fig 8

Unfixed, unglycerinated cortical fiber cells of the 80 year old human lens show aggregates of chains of protein (open arrowhead) but no 10 nm filaments 84 000  $\times$ .

highly coiled and aggregated. The chains were never identified in the cells. Whether the backbone of the chains is actin remains to be elucidated.

Although the number of lenses in this study is small, the observation that intermediate filaments are concentrated in the epithelial and superficial cells is consistent with previous observations (Masei 1977, Bradley *et al.* in press). The role of the intermediate filaments in eukaryotic cells is poorly resolved. A cytoskeletal function has been postulated as well as a role in anchoring of the nucleus (Lehto *et al.* 1978). Kuwabara (1975) pointed out that the lens appears to change its shape during accommodation mainly by changing cellular shape in the superficial fiber zone. The relationship of the intermediate filaments and actin filaments in this process requires further study. Rafferty & Gaffney (1978) suggested that the role of cytoplasmic filaments is either to support a spherical shape or to provide a contractile force during the process of accommodation. Whether loss of filaments is involved in the loss of accommodative ability with aging or in the cataractous process remains to be explored.

### Acknowledgment

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# FIBRINOLYTIC FACTORS IN AQUEOUS HUMOUR AND SERUM FROM PATIENTS WITH FUCHS DYSTROPHY AND PATIENTS WITH CATARACT

BY

THORKILD BRAMSEN and STENER STENBJERG

The concentration of  $\alpha_2$  macroglobulin,  $\alpha_1$  antitrypsin, plasminogen,  $C_3$  complement, fibrinogen, degradation products (FDP) and fibrinolytic activity were studied in the aqueous humour and serum from six patients with Fuchs endothelial dystrophy, 17 patients with uncomplicated senile cataract and in the secondary aqueous from six cataract patients. Finally the aqueous humour and serum from two patients with anterior uveitis were studied.

An increased concentration of  $\alpha_2$  macroglobulin,  $\alpha_1$  antitrypsin, plasminogen and  $C_3$  complement was found in both the aqueous and the serum from patients with Fuchs dystrophy when compared with the primary aqueous and serum from patients with cataract, but this was only significant for  $\alpha_1$  antitrypsin in aqueous humour. A significant increase in the amount of FDP was found in the serum of the Fuchs patients compared with the cataract patients. Fibrinolytic activity could not be demonstrated in the serum in any of the patient groups.

The concentrations of the various factors found in the secondary aqueous of the cataract patients differed only slightly from the content of the primary aqueous of the Fuchs patients.

**Key words:** Fibrinolysis - tranexamic acid - Fuchs endothelial dystrophy - cataract - uveitis anterior - aqueous humour - complement.

nsen & Ehlers (1977) found that the antifibrinolytic agent tranexamic acid has a reducing effect on the corneal oedema occurring in Fuchs dystrophy. It is therefore considered relevant to examine certain of the factors in the fibrinolytic system in patients with this disease.

Corneal oedema occurs in connection with inflammatory conditions in the anterior segment of the eye. Measurable oedema is always present in acute anterior uveitis (Mishima 1968). This oedema can occasionally be seen in the form of folds in Descemet's membrane. The oedema which occurs on the onset of an anterior uveitis can be assumed to be secondary to the inflammatory condition in the uvea.

It was demonstrated in a doubleblind trial (Bramsen et al 1978) that tranexamic acid was able to reduce the corneal oedema occurring after cataract operations. One possible explanation is that the secondary aqueous contains factors which either cause or maintain the oedema and that these factors could be affected by an antifibrinolytic agent.

On the basis of the supposition that the aqueous humour in patients with Fuchs dystrophy in anterior uveitis and following operative intervention in the anterior chamber contains factors which are responsible for the corneal oedema, it was decided to examine certain of the factors connected with the fibrinolytic system and complement system in the above mentioned groups of patients and in patients without corneal oedema.

## Material

The material consisted of nine patients with Fuchs endothelial dystrophy and cataract. The average age was 69.3 years with a range from 56 to 80 years. A second group consisted of 17 patients in which senile cataract was the sole eye disease. In this group the average age was 73.8 years with a range from 53 to 94 years. Finally there were two patients with anterior uveitis, both of whom had corneal oedema with normal intraocular tension. The patients with Fuchs dystrophy and the cataract patients were referred with regard to cataract operation. The uveitis patients were seen because of recurrent inflammation complicated by cataract with visual impairment.

## Methods

All of the operations were performed under retrobulbar anaesthesia. Prior to operation 2.0 ml of aqueous was aspirated. Venepuncture was performed immediately after this. Two aspirations were undertaken in six of the 17 cataract patients. Approximately 10 min elapsed following removal of the primary aqueous until the anterior

chamber was reformed and in these six patients the reformed aqueous was (approx 100  $\mu$ l)

Serum and aqueous humour were applied without delay to the fibrin plate. The test material was frozen at  $-20^{\circ}\text{C}$  for rocket immunoelectrophoresis and for titration. Special tubes containing thrombin and epsilonaminocaproic acid were used for blood samples intended for FDP quantitation.

Fibrinolytic activity was estimated by the fibrin plate method (Astrup *et al.* 1952). 30  $\mu$ l fresh aqueous humour or serum was applied to the surface of the plate. After 18 h of incubation at  $37^{\circ}\text{C}$  the plates were inspected for areas of lysis.

$\alpha_2$  macroglobulin,  $\alpha_1$  antitrypsin, plasminogen,  $\text{C}_3$  complement were quantitated by rocket immunoelectrophoresis in which the protein was precipitated in an agarose gel containing the specific antibody. The size of the rocket is directly proportional to the concentration of the antigen in question (Laurell 1966). Antibodies were purchased from Behringwerke, W. Germany. The results were expressed as per cent of concentration found in pooled normal serum from healthy donors 10 men and 10 women.

Serum samples were applied in a 1:20 dilution while the aqueous humours were tested undiluted to bring the protein concentration within the discriminating range of the method. In our hands the method had a coefficient of variation of 9%.

Fibrinogen degradation products (FDP) were measured by the Thrombolytic test using latex particles coated with antihuman fibrinogen (Wellcome Laboratories, Beckenham, England). The test is semiquantitative. The serum was diluted 1:10. The test agglutination occurs if the concentration of FDP exceeds 0.5 mg/l. The test was tested undiluted.

## Results

The content of the various factors in the aqueous is shown in Table I. It can be seen that the concentration of  $\alpha_2$  macroglobulin is almost the same in the aqueous from patients with Fuchs' dystrophy, uveitis and in the secondary aqueous from the cataract patients whereas it is only about half of this in the primary aqueous of the cataract group. The difference between the aqueous from the Fuchs' patients and the primary aqueous from the cataract patients is however not significant ( $t = 1.6$ ).  $\alpha_1$  antitrypsin is found to be increased in patients with Fuchs' dystrophy in uveitis and in the secondary aqueous from the cataract patients. The difference between Fuchs' dystrophy and the primary aqueous from cataract patients is in this case significant ( $t = 2.9$ ,  $P < 0.05$ ). The concentration in the secondary aqueous from the cataract patients is between that of the primary aqueous and the aqueous concentration from the Fuchs' dystrophy patients. The concentration in the patients with uveitis is approximately 9 times more than the primary aqueous of the cataract patients.

Table I

relative concentrations in aqueous humour of factors of the fibrinolytic system—complement factor C<sub>3</sub> as determined by immunoelectrophoresis using pooled normal serum as reference.

	Fuchs dystrophy	Cataract Primary aqueous humour	Cataract Secondary aqueous humour	Anterior uveitis
acroglobulin	0.14	0.09	0.11	0.15
SEM	0.04 (n = 9)	0.01 (n = 17)	0.02 (n = 6)	0.05 (n = 2)
trypsin	1.90	0.30	0.67	5.60
SEM	0.36 (n = 9)	0.04 (n = 17)	0.18 (n = 6)	2.40 (n = 2)
fibrinogen	0.41	0.07	0.28	2.85
SEM	0.09 (n = 9)	0.01 (n = 17)	0.16 (n = 6)	0.45 (n = 2)
complement	0.19	0.09	0.25	1.70
SEM	0.06 (n = 8)	0.02 (n = 16)	0.05 (n = 9)	0.30 (n = 9)
	negative (n = 8)	negative (n = 16)	negative (n = 9)	negative (n = 2)
in plates	negative (n = 8)	negative (n = 16)	negative (n = 9)	negative (n = 9)

Plasminogen is increased in the Fuchs dystrophy patients in anterior uveitis and in the secondary aqueous of the cataract patients. The concentration in the secondary aqueous also lies between that of the primary aqueous of cataract patients and the aqueous from patients with Fuchs dystrophy. The difference between Fuchs and the primary aqueous from cataract patients is not significant ( $t = 1.54$ ).

C<sub>3</sub> complement is also increased in the patients with Fuchs dystrophy in anterior uveitis and in the secondary aqueous of cataract patients. The difference between the primary aqueous from cataract patients and the aqueous from Fuchs patients is not significant ( $t = 1.58$ ).

Neither FDP nor fibrinolytic activity could be demonstrated in any of the samples.

The serum content of the various factors is shown in Table II. It is to be noted that all the patients underwent venepuncture only once. The serum



values for the six patients in whom secondary aqueous was removed, therefore also included in the values for the primary aqueous group.

In the case of  $\alpha$  macroglobulin and  $\alpha_1$  antitrypsin it can be seen that the values are almost identical for the cataract group and the uveitis patients, whereas a non significant tendency towards higher values is found in patients with Fuchs dystrophy.

In the case of plasminogen and  $C_3$  complement higher concentrations were found in patients with Fuchs dystrophy and anterior uveitis than in patients with cataract (not significant).

FDP could be demonstrated in the serum ( $> 10$  mg/l) in six out of the patients with Fuchs dystrophy. In the cataract group FDP was demonstrated in three out of 16 patients. This difference is significant (Fischer  $P < 0.05$ ). Fibrinolytic activity could not be demonstrated in the serum in any of the patient groups.

Table II

The serum concentration of various factors of the fibrinolytic system expressed as percentage of the normal serum concentration

	Fuchs dystrophy	Cataract Primary aqueous humour	Cataract Secondary aqueous humour	Reference
$\alpha$ macroglobulin	114.1	96.2	93.3	91.0
$\bar{x} \pm \text{SEM}$	8.9 (n=8)	10.3 (n=16)	10.9 (n=6)	10.1 (n=16)
$\alpha_1$ antitrypsin	96.2	91.1	90.6	87.5
$\bar{x} \pm \text{SEM}$	5.3 (n=8)	3.4 (n=16)	5.5 (n=6)	3.3 (n=16)
Plasminogen	71.1	66.0	67.8	50.1
$\bar{x} \pm \text{SEM}$	6.5 (n=8)	3.1 (n=16)	6.4 (n=6)	1.0 (n=16)
$C_3$ complement	123.2	115.5	107.4	113.5
$\bar{x} \pm \text{SEM}$	6.5 (n=8)	3.5 (n=16)	6.6 (n=6)	3.5 (n=16)
FDP ( $\geq 10$ mg/l)	positive 11 negative 2	positive 3 negative 13	negative 6	negative 17
Fibrin plates	negative 8	negative 16	negative 6	negative 17

## Discussion

There are no studies in the literature concerning the concentration of the factors mentioned in the aqueous humour of humans. It is therefore somewhat questionable to consider the aqueous of cataract patients as being normal. For practical reasons this is the most normal material that can be obtained. In the present study a comparison is made between cataract patients and patients with Fuchs dystrophy and as described cataracts were also present in the Fuchs patients. It must therefore be reasonable to relate the difference in the composition of the aqueous to the difference between cataract patients and patients with Fuchs dystrophy. The rocket immunoelectrophoresis is sensitive to protein concentrations down to 0.3 mg/l. Serum samples were diluted 1:20 in water before application while the aqueous was tested undiluted. This means that concentrations down to 0.05 % of serum values could be examined with accuracy. The difference in the aqueous between these two groups of patients (significant for  $\alpha_1$  antitrypsin) could be due to the effect of the pathological endothelium of the Fuchs patients. However it is also possible that the aqueous of Fuchs patients has a toxic effect on the cornea endothelium. The last statement appears to be confirmed by the composition of the aqueous in the patients with uveitis. In uveitis there is inflammation of iris and ciliary body which has a breakdown of the blood aqueous barrier and cornea oedema is seen to develop in these patients. The alteration in the examined factors in the aqueous is greater in the patients with anterior uveitis than in the patients with Fuchs dystrophy. In anterior uveitis the oedema develops acutely as compared to the oedema in Fuchs dystrophy which develops over the course of several years. The patients with anterior uveitis had an increased level of complement in the serum (not significant) whereas the other factors in the serum did not differ from the patients with cataract. FDP was negative in these patients. This suggests a local reaction in the anterior segment of the eye. In the Fuchs patients there was a tendency towards a higher serum concentration of the factors examined and there was a significantly increased FDP concentration. This could be explained if Fuchs endothelial dystrophy occurs as a part of a generalised disease with alterations in the fibrinolytic system. In this way the reducing effect of tranexamic acid on corneal oedema in Fuchs dystrophy is understandable. The present study suggests that alterations in the composition of the aqueous for local or more general reasons can cause overcompensation of the cornea endothelium. This situation must for example be considered when placing clip lenses in younger patients.

## Material and Methods

Sixteen cataractous lenses extracted from eyes with clinical FEC syndrome and cataractous lenses from otherwise normal eyes were subjected to scanning electron microscopy and confirmatory transmission electron microscopy after having been treated to a variable degree of either  $\alpha$  chymotrypsin or collagenase or a combination of both. Some lenses were untreated and some were sham treated serving as experimental controls (Table I).

Following extraction the lenses were immediately put into a small beaker containing sterile perfusion medium without enzymes. The lenses were transferred with a pair of forceps to a platinum grid and perfused with enzyme solution at a rate of one drop per second by means of a peristaltic pump. The whole experiment was carried out in an incubator at 37°C and lasted from 45 to 120 min. Following the perfusion, the lenses were washed for 24 h at 5°C in 0.1 M 5% glutaraldehyde buffered to pH 7.4 with HEPES buffer. One slice was then cut and processed according to earlier description (Seland 1971) and the two remaining parts were bisected producing four quarters of the lens. Two quarters were processed for SEM according to an earlier described method (Seland 1971). TEM sections were cut in an LKB microtome and examined in Philips EM 300 microscope while the tissues for SEM were processed through a critical point apparatus (Baltec) and coated with approx 17 nm gold in a sputter apparatus (Polaron) (Reli & Fiedler 1971) and examined in a Philips PSEM 500 microscope.

The following enzyme concentrations were used:  $\alpha$  chymotrypsin (Novol 400 units per 100 ml perfusion fluid) and collagenase (Sigma type III and V) 400 units per 100 ml perfusion fluid. The perfusion fluid consisted of Ringer acetate buffer pH 7.4 with HEPES buffer and 4 mg gentamycin (Garamycin®) added per 100 ml.

### Control lenses

Two lenses with clinical fibrillography (Nos 1 and 2) and two lenses with senile cataract (Nos 3 and 4) were processed for microscopy without having been subjected to any perfusion experiments. In addition two lenses extracted from normal eyes (Nos 5 and 6) and two cataractous lenses (Nos 7 and 8) were subjected to perfusion for 60 to 90 min in an experimental procedure identical to the rest of the lenses but without addition of any kind of enzymes. These eight lenses were considered as control group.

### $\alpha$ chymotrypsin experiments

Two FEC lenses (Nos 9 and 10) and two normal cataractous lenses (Nos 11 and 12) were subjected to enzyme action for 60 to 90 min.

### Collagenase experiments

Three FEC lenses (Nos 13–15) and three normal cataractous lenses (Nos 16–18) were subjected to collagenase actions for periods varying from 60 to 90 min.

### Combined enzymatic action

Seven FEC lenses (Nos 19–25) and three normal lenses (Nos 26–28) were subjected to a perfusion with a combination of  $\alpha$  chymotrypsin and collagenase for 45 to 90 min.

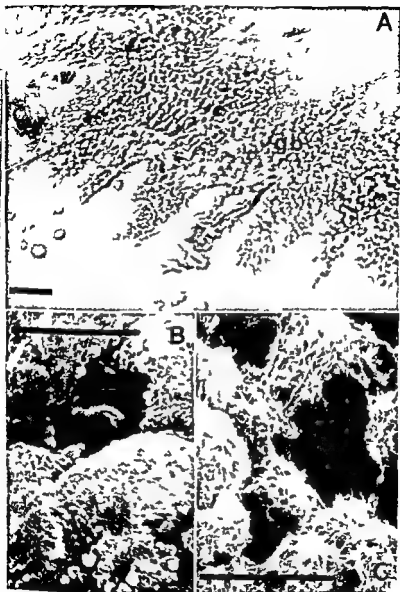


Fig 1

Anterior surface of untreated FEC control lens

The peripheral granular band (gb) is seen as an irregular layer in the periphery

Anterior pole towards upper right corner Bar 100  $\mu$ m

and C. Busacca bushes from granular band of two different untreated FEC lenses  
B there is a flattened appearance of the fibrillar network with small melanin  
derules (arrows) and smooth red blood cells In C the Busacca bushes have a highly  
fibrillar appearance Bars 10  $\mu$ m



Fig 2

**A Sham treated lens**

Section through lens capsule in the area of zona germinative. Highly porous area of epithelial cells (E) with overlying lens capsule (lc) containing fibrous deep folds (d) with tentacles and inclusion bodies. Bar 10  $\mu$ m

**B Phasecontrast of semithin section from zona germinative after collagenase treatment**  
Only remnants of capsula propria (arrow) are seen on an intact discoid plane of the layer (d) E- epithelial cell Bar 10  $\mu$ m

## Results

## Control experiments

Major differences could be found between the surface ultrastructure of the untreated and sham-treated lenses. They had a similar distribution of fibrolopathia material on the anterior lens surface with a central disc and a peripheral granular band (Fig. 1). The central discs however were not fully conspicuous in all lenses. The FEC material was usually rich on the zonular threads in the equatorial region. In the sham group the anterior capsule had occasionally linear breaks in the equatorial region which might be discoid plaques of deep layer with pits and tentacles (Fig. 2 A) (Seland *et al.*). Large magnifications of the surface material from the peripheral band and central discs in FEC lenses showed a strikingly similar intertwined fibrillar structure (Fig. 12 A B C D). The material in the peripheral granular band differed only from the central disc by increased presence of spherical melanin granules (Fig. 1 B). The thickness of the fibrillar components were measured as 50–80 nm in the SEM preparations. The lengths were difficult to judge as the fibrils were twisted in a complex meshworks but at least 3  $\mu\text{m}$  could be discerned.

The non-FEC lenses did not show any of the above mentioned surface features. The zonular threads were as a rule well preserved both in untreated and sham-treated lenses.

## Chymotrypsin experiments

Neither the FEC material from central disc nor the peripheral band were apparently affected by  $\alpha$ -chymotrypsin (Fig. 3 A). The zonular threads covered with FEC material were also more resistant to the enzyme action than normal granules (Fig. 3 B).

In the normal lenses 90 min of perfusion left a completely smooth and bald surface with no traces of zonular apparatus but the different textures of the anterior and posterior capsules were separated by a distinct equatorial line.

## Collagenase experiments

The collagenase-treated FEC lenses had an intact peripheral band but no central disc on the anterior capsules (Fig. 4 A). The zonular apparatus could be found unaffected while the capsula propria centrally and between the zonular threads was heavily eroded (Fig. 4 B). In lens no. 13 a linear rupture in the zonular lamina had occurred in the preequatorial region and the enzyme had gained access to the underlying capsula propria digesting the superficial part and revealing large circular areas with diameters varying from 100–150  $\mu\text{m}$ .



Fig 3

*a* chymotrypsin experiment

- A The peripheral granular band is not removed by the enzyme  
B FEC material seem to protect some zonal threads from the action of the enzyme

Anterior poles towards left Bars 100  $\mu$ m

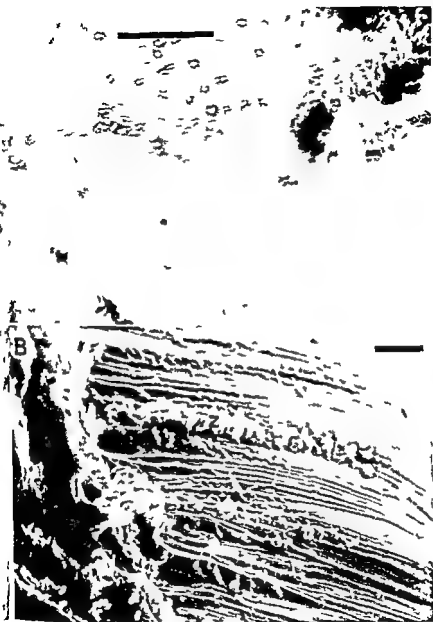


Fig 4

Collagenase experiment

- A The peripheral granular band (gb) = unaffected by enzyme Bar 100  $\mu$ m Inset  
Enlargement of the Busacca bushes shows a normal fibrillar structure Bar 1  $\mu$ m
- B Zonular threads (z) are not destroyed by collagenase Bar 100  $\mu$ m



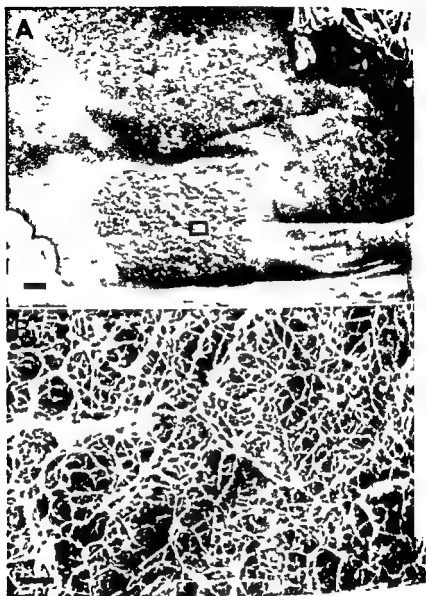


Fig 5

Collagenase experiment

- A Preequatorial region where zonular lamina has ruptured. Circular area of intertwined fibrils. Bar 10  $\mu\text{m}$
- B Enlargement of rectangular area in A. The fibrils have a thickness up to 0.22  $\mu\text{m}$  and length up to 3  $\mu\text{m}$ . Bar 1  $\mu\text{m}$

responding in site and size to large discoid plaques of deep layer (Fig 5 A) with magnifications of their surface revealed an intricate configuration consisting of interwoven fibrils with diameters from 50–80 nm (Fig 5 B and C). Thin and ultrathin sections through these circular areas in FEC lenses confirmed that they represented discoid plaques of deep layer with intact fibrils and a variable degree of residual capsule (Fig 2 B). In the  $\alpha$  chymotrypsin treated lenses the normal cataractous lenses in the trypsinase experiments had a clearly defineable equator parallel demarcation



*Fig 6*

Combined enzymatic experiment on FEC lens  
discoid plaque sector (cr) in the anterior lens capsule ending in a distinct laminar  
edge (c) Bar 100  $\mu$ m

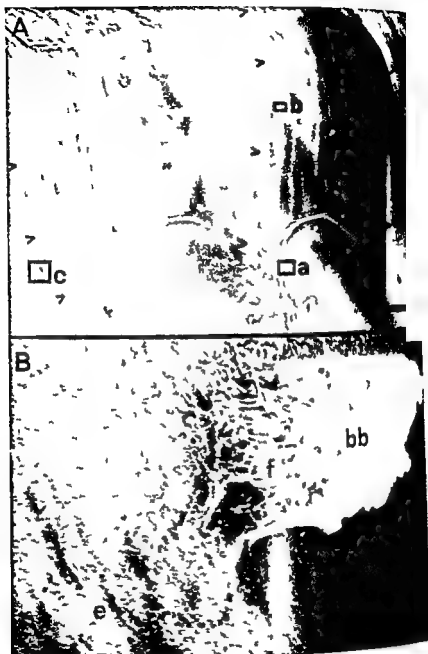


Fig 7

Combined enzymatic experiment on FEC lens

- A Anterior lens capsule with three cobble stone sectors (arrows) terminal projections a b c Enlarged areas Bar 100 μm.
- B (box a in fig 1 A) Busacca bush (bb) at the edge of the lamina Note the structure (f) of the bush and its connection to the cobble stone elevation (e) Enlarged area



*Fig 8*

Combined enzymatic experiment on FEC lens

Enlarged section from fig 7 A

- (box b) Midsection of cobble stone sector Note the cobble stone (e) parallel linear structures (f) and the globular formations (arrows) Bar 10  $\mu$ m
- (box c) Large elevations (e) on the edge of the lamina surrounded by finely fibrillar material (f) Bar 10  $\mu$ m

be separating the anterior lens capsule from the posterior Remnants of the  
ular threads were also observed The hexagonal outline of the lenticular  
ls could easily be depicted through the remaining thin film of capsular  
aterial in some lenses

Ehlers 1969) The  $\alpha$  chymotrypsin easily degrades the normal zonular threads heavily infested with IEC material however were not affected by the enzyme. Whether the IEC material constituted a barrier or the zonular apparatus had a pathological structure cannot be determined. Davanger & Pedersen (1975) have demonstrated that Busacca bushes constitute a considerable barrier for large molecules and therefore not inconceivable that they may afford a true protection against enzymes.

The experiments also showed that the central discs were readily removed by collagenase acting on the underlying capsule. In addition to the observation that no characteristic changes have been described in the central capsule propria this is strong evidence for the view that the central disc is probably only a secondary phenomenon caused by the material being transported to this location by the aqueous humour. The peripheral granular band was however unaffected by any single enzyme signifying a strong anchorage.

The peripheral granular band and the zonular excrescences were both removed by a relatively short concerted action of both enzymes leaving the peripheral edge of this band which appeared as a serrated lamina.

In this relatively superficial layer of the lens capsule several distinct types of enzyme resistant capsular inclusions were demonstrated.

The most conspicuous were smooth rounded cobble stone elevations with diameters from 2 to 12  $\mu\text{m}$  which were arranged in radial sectors and teeth like projections of the laminar edge. These elevations probably represent some of the large inclusions in the IEC lens capsule earlier described as and dots (Bertelsen et al 1964).

The two other formed bodies observed in this layer were also present in a more conspicuous way in the epithelium near stratum where relatively worm like spindleshaped structures with diameter of about 800 nm and length exceeding 3  $\mu\text{m}$  were found. The only difference between the appearance of the superficial cobble stone stratum and the deep epithelium near stratum was a high degree of directional uniformity in the former where the excrescences were parallel and radially arranged (Fig 8 B). The structures found in the capsule were curved in a haphazard fashion (Fig 9 A). These structures probably represent the normal capsular inclusion bodies found with increasing age in all capsules (Dark et al 1969 Seland 1974). The superficial spindleshaped structures may possibly afford the necessary deep anchorage or precipitation cores for the fine fibrils of the Busacca bushes. Their uniform orientation may be explained as a result of the centripetal growth of the capsule. On the other hand they might represent an artifact.

ird conspicuous component of the FEC capsules were small globular res seen both in the superficial layer and in the epithelium near stratum probably represent the annular bodies demonstrated in the capsula (Bertelsen et al 1964)

ly fibrillar material (FEC material) could be identified on the lens (Fig 12 A B C D) and in the superficial cobble stone stratum (Fig s well as a component of the deep layer of discoid plaques (Fig 9 A E and G) The fibrils at all sites had dimensions of the same order of tude i.e. about 50–80 nm thick and length varying up to 5  $\mu$ m The st concentrated area found to be covered by the fibrillar surface of a d plaque was about 10  $\mu$ m corresponding to a cell diameter (Fig 9 A) g into consideration a coat thickness of 15–20 nm they correspond to the opathy material observed earlier (Bertelsen et al 1964 and Sugar et al

some lenses wells and holes could be found in the preequatorial i with small rounded structures inside (Fig 11 A) These probably repre he Hassall Henle like warts sometimes found in this region (Seland

ed on the present findings the phenomenon of FEC may be explained in llowing way

e to some unknown stimulus cells in the zona germinativa of the lens forming pathological fibrillar material In addition the cells are stimulated oduce increasing amounts of the coarser formed bodies of the normal lens capsule As the major part of the capsular production occurs in the germinativa and as a continuous resorbtion probably occurs in the central ot covered by lamina zonularis the growth turnover results in a sliding port of capsule from the production site towards the resorbtion area ng with it all kinds of inclusions and pathological capsular substances d 1976) As long as production of pathological capsular components is the growth transport will remove all pathological components from the elium and in due course release it on the surface of the lens If the logical fibrillar production is accelerated large aggregation will form on roduction site in the shape of discoid plaques of deep layer

production of pathological capsules seem to occur in 20–30 preequatorial m the concentrating effect of the centripetal transport may cause the logical capsular substance to form preequatorial stria (Bartholomew 1971) a sheath or lamina when it appears in the smaller resorbtion area e resorbtion process of the aqueous humour aided by the mechanical action e pupillary border releases the capsular components Some fibrils how have firm anchorage in capsular meshwork or to the coarser spindle

Ehlers 1969) The  $\alpha$  chymotrypsin easily degrades the normal zonular threads. Zonular threads heavily infested with FEC material however were not affected by the enzyme. Whether the FEC material constituted a physical barrier or the zonular apparatus had a pathological structure, is not determined. Davanger & Pedersen (1975) have demonstrated that the Busacca bushes constitute a considerable barrier for large molecules and therefore not inconceivable that they may afford a true protection against enzymes.

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*Short communication*

**VITREOUS CUTTER IN ANTERIOR SEGMENT SURGERY**

BY

**MARTIN LOWES and NIELS VEDEL-JENSEN**

Experience has been gained in operating upon a variety of anterior segment disorders using a Grieshaber vitreous cutter. The results and complications are briefly discussed.

*Key words:* vitreous cutter - lensectomy - anterior segment surgery

In 1971 Machemer introduced the vitreous infusion suction cutter (1) whereby ocular tissue could be cut, removed and replaced through a tube inserted via the pars plana. The technique was primarily used for use in the posterior segment of the eye (Machemer et al 1971) but has also been applied in a wide variety of pathological conditions in the anterior segment of the eye with favourable results (Hanski & Crick 1971, Tamm Machemer 1978, Lullwitz & Sautter 1978). Our experience with this technique in 19 patients with various anterior segment disorders is presented.

**Material and Methods**

A total of 19 patients underwent surgery and of these there were seven with cataract including three cases of congenital cataract, two cases of traumatic cataract, one case of subluxated congenital cataract and one case of secondary cataract following chronic anterior uveitis. Nine cases underwent anterior segment removal either for removal of pupillary membranes, removal of capsulo-lenticular membranes for pupillotomy. There was one case of vitreous opacities following retinal detachment extraction and two cases of aphakic bullous keratopathy.

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*Operative technique* The operation was performed under the operating microscope using a Greishaber vitreous cutter the cutting head of which consists of a rotatory millating four bladed knife contained within a metallic tube. The material to be cut is aspirated through a small hole directly adjacent to the knife blades. Inflow of fluid into the anterior chamber occurs through the central tube and is controlled by the surgeon. The anterior chamber was opened at the limbus and the vitreous cutter inserted while keeping the incision as watertight as possible. The cutting was controlled by the surgeon and aspiration performed by the assistant. In the two cases of traumatic cataract and in eight of the nine cases of anterior segment reconstruction the operation was performed as a new technique designed to remove the scaffolding which had previously caused repeated opacification following operations with a dissection knife or Vannas scissors.

## Results

*Lensectomy* The technique proved to be satisfactory in removing lenticular tissue especially when this was soft. In the case of the secondary cataract the lens nucleus was too hard to be aspirated and the incision was therefore extended and the nucleus removed by extracapsular extraction. Improvement in visual acuity occurred in all seven cases. The preoperative visual acuity was 0.9 or less in all cases. The postoperative visual acuity was 0.3 in three cases and better than 0.5 in the remaining four cases. Transient glaucoma and permanent sector striate keratitis occurred in one case of traumatic cataract but probably due to prolonged operative intervention. The eye has since obtained a visual acuity of 1.0.

*Anterior segment reconstruction* This was undertaken in nine eyes including seven cases with capsulo lenticular remnants and two cases with pupillary membranes where pupil enlargement was performed. In two cases the pupillary membranes were of such a thickness that they could not be directly aspirated into the cutting port and had to be cut into smaller pieces using Vannas scissors. The preoperative visual acuity was counting fingers or less in six cases, 0.1 in two cases and 0.4 in one case. Postoperatively considerable improvement had occurred in five cases with a visual acuity of 0.3 in three cases, 0.5 in one case and 0.7 in one case. In the remaining four cases visual acuity was more or less unchanged and retinal detachment occurred in two of these cases. Transient striate keratitis occurred in four eyes. In one eye a thick fibrous membrane could not be removed.

*Bullous keratopathy* In the two cases of bullous keratopathy operation failed to remove the cornea oedema. One case developed a transient glaucoma.

*Vitreous opacities* In the sole case of aphakic vitreous opacities anterior vitrectomy resulted in a visual acuity of 1.0.

## Discussion

The use of the vitreous cutter provides an interesting new development in surgery of the anterior segment of the eye. Tissue is removed in small pieces under microscopic control and the anterior chamber is maintained throughout the procedure. In the present series the technique was successfully used in 15 out of 16 cases of cataract and anterior segment reconstruction. In 11 cases where the lens was hard or the membranes were thick, no difficulty was experienced with the rotatory cutter. This led to 10 operations with excessive fluid inflow. This undoubtedly caused to cell damage with subsequent transient striate keratitis in four cases and permanent sector striate keratitis in one case. The two cases of transient seemed to occur on the basis of a type of malignant glaucoma and due to excessive fluid forming pockets in the vitreous. Retinal detachment occurred in two cases. In this respect retinal detachment was also reported by Litwin (1978) following the use of the Kaufman vitrector I where the action led to spooling of the vitreous. The instrument has since been modified and the Kaufman II has a guillotine action. In our opinion this constitutes a significant advance in anterior segment surgery. However, the rotatory action is not effective enough for anterior segment surgery. The complications encountered are to a large extent felt to be due to the inefficiency of this rotatory action. Accordingly a further series is planned with a vitreous cutter with a guillotine action.

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Short communication

# FLICKER COMPARISON OF FUNDUS PHOTOGRAPHS

A technical note

BY

BO BENGTSSON and C E T KRAKAU

A method for comparison of two fundus transparencies by means of alternating flickering light is described. Spurious differences between the pictures are reduced by making the exposures in a fixed position of the pulse cycle. Small haemorrhages are readily detected. Pulse variations of the vessels and changes in size of cup and disc can be measured.

*Key words:* papillary measurements — pulsations

Repeated photography of the eye fundus is a method to be recommended when development of pathological processes has to be followed but subtle changes difficult to estimate by simple examination of the pictures. The method called Stereochronoscopy developed by Goldmann & Lotmar (1977) deals with the problem of comparing fundus photographs taken on different occasions.

The method to be described here is intended not only to make possible the observation of minute changes between pictures but also to measure them. The principle adopted is well known from other fields such as astronomy and cinematography (Edholm 1976).

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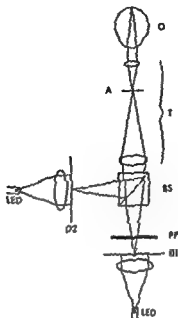


Fig 1

Flicker comparator O observer's eye T magnifying tube BS beam splitter PP parallel glass plate D1 D2 transparencies LED light emitting diodes A focusing lens

### Method

The two transparencies to be compared are fitted into an instrument as shown in Fig 1. They are illuminated from behind and by use of a beam splitter they are seen superimposed and magnified 10-20 diameters in the tube. One of the pictures is placed on a cross table, the other mounted on a rotating setting and it is therefore possible to adjust identical pictures in order to be seen in complete coincidence through the tube. In order to detect discrepancies between pictures taken on different occasions of the same ground the light sources which are light emitting diodes (LEDs) are used, which operate at frequencies which can be set from 1/2 Hz to about 20 Hz. The LEDs have changed position somewhat between the two occasions and are seen fluttering whereas the rest of the picture remains stable.

In order to make small deviations measurable a plane parallel transparent glass plate (PP) is mounted between one of the pictures and the beam splitter. This can be rotated around a horizontal axis thus producing a lateral displacement of one image. The relation between the rotation of the mirror and the lateral placement of the image is approximately linear for small angles.

The light intensity of the LEDs can be balanced so as to compensate for differences in exposure

#### Sources of spurious variation

Various sources of error which may cause spurious differences between the pictures are discussed by Goldmann & Lotmar (1978). In order to avoid parallax three dimensional structures for example excavated discs have to be photographed from the same direction and through the same part of the optic media each time. By using the procedure for photography through a natural pupil (Engtsson & Krakau 1979) these conditions are largely fulfilled. Centering the pupil in relation to the camera and a correct distance to the eye are the essentials of this technique.

The direction of the gaze is checked by the location of the disc on the pictures.

#### Measurements

For the study of the pulsatile variations a device was constructed by means of which an exposure can be made at a predetermined moment of the pulse cycle. A small ECG amplifier is connected to the patient and the R peak is allowed to start a time circuit which in its turn determines the moment when it is possible to release the flash. Thus when the exposure lever is pushed the camera shutter is opened but the flash is not released until a pre set delay (0.1 to 1 sec after an R peak) has elapsed.

### Discussion

Series of pictures were taken on a normal person with clear mediae keeping the adjustment as constant as possible and varying the moment of exposure we could see the configuration variation of the vessels at the papilla when comparing pictures in different pulse phases. Displacements can be determined on sharp pictures with an s.d. of  $< 2\mu$  a degree of precision which makes the study of pulsation effects possible. The veins compressed during systole dilated to maximal diameter about 0.4 secs after the R peak. The amplitude was of the order  $40\mu$ . The artery showed caliber changes too but to a smaller degree – about  $5\mu$ . There was also serpentine movements – The measurements were made at a flicker frequency of 1–5 Hz.

On Glaucoma cases haemorrhages occurring on the papilla were detected without difficulty. A haemorrhage present on one picture but not on the other is very striking coming and going with flickering of the lights.

Also the extension of the excavations can be detected. Small distinctly demarcated vessels on the papillary substance are landmarks.

Some small errors of adjustment (or by change of position between adjustment and exposure) causing differences between pictures can seldom be completely avoided. Since parallax errors displace corresponding points in the same direction which is not the general rule in the case of true changes in shape it may be possible to avoid spurious differences by careful examination. The use of more than one exposure on each occasion so that points of best correspondence can be chosen is also of value at evaluation. Furthermore a series of exposures locked in a fixed pulse phase is recommended in order to detect very small changes. A material of repeated exposures in the fixed pulse phase on increasing glaucomatous excavations is now being collected with the aim of elucidating the efficiency of the method in practice.

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## JUDICIA DE NOVIS LIBRIS

*Harold A & Slatt Bernard J* The Ophthalmic Assistant Fundamentals and clinical practice 3ed pp 594 853 ill Mosby St Louis 1976 Price \$ 24 95

The new edition has been expanded with two chapters concerning soft contact lenses and questions after each chapter actual new topics such as night vision goggles in retinitis pigmentosa prosthetic aids for the blind (TV cameras under motor control) intraocular lenses phacoemulsification automatic refractors AO non contact meter computer systems for hard contact lens fitting hard gas permeable contact lens pilocarpine spray bottle and ocusert

The text is easy to understand vivid and interesting The artistic figures and photographs are very instructive The book covers all aspects of interest for the ophthalmic assistant inclusive clinical practice tonography eye photography optic illusions pharmacology refraction contact lenses eye disorders classification of urgency aseptic technique and glossary etc

The atlas of common eye disorders unfortunately only consists of black and white photographs

In the next edition a chapter about the preparation of a scientific paper could perhaps be added

The book can be warmly recommended to any ophthalmic assistant

W S Vorn

*Hubert Babel Nicolas Stangos Silvio Korol & Micheling Spiritus* Ocular Electrophysiology A Clinical and Experimental Study of Electroretinogram Electro Oculogram Visual Evoked Response Georg Thieme Publishers Stuttgart 1977 172 pages 145 Figures 21 Tables Price Da kr 264 00

In the short introduction the authors stress the difficulties which have arisen especially from the clinical view point in registering from a localised zone and in attempting to attain standardization of the methods of examination

The authors have since 1968 developed and standardized a reproducible technique for electrophysiological registration by means of a computer both in normal and in pathological cases

The book has four chapters and the clinical examinations of electroretinography (ERG) electrooculography (EOG) and visual evoked response (VER) are individually discussed in separate chapters The fourth and last chapter deals with experimental investigations concerning the correlation between morphology and electrophysiology Each of the three clinical chapters on ERG EOG and VER have the same composition in that they begin with a description of the background for the electrophysiological registration The authors then describe their own technique and the results obtained when examining normal subjects

Furthermore each chapter has a section showing examples of pathological ERG EOG and VER in various diseases of vascular degenerative and inflammatory nature in the retina pigment epithelium and the optic nerve This is further illustrated by case reports fluorescein angiograms drawings of the pathological registration and a com-



parison of the results which can be obtained in the individual case by electroretinography or electrooculography. In the fourth and final chapter experimental examinations the effect of intravenous or intravitreal injections of substances (ex. monoiodacetate, ferro oxide, xylocaine and glycine) on the potentials is shown. By means of electronmicroscopy the nerve or cell lesions caused by the toxic substances are shown and in this way it is possible to determine the origin of the abnormal registrations in clinical electrophysiology.

Even though the book deals with an extensive subject - clinical electrophysiology - in comparatively few pages it does however manage to be comprehensive and informative. It is easily read and answers many questions. It can be warmly recommended.

 $\Sigma F_x = 1$ 

*Documenta Ophthalmologica Proceedings Series* vol 11 With ISCEERG Symposium kibbutz Ginosar Israel 1975 *Experimental and Clinical* 1:2 (Eds Auerbach & Henkes) 1977 Pp 207 89 Figs 8 Tables.  
Price Dutch Glds 60- ISBN 90 6193 151 7

This volume presents basic and clinical studies of amblyopia and other aspects of the visual system. Research in the field of normal visual physiology is also covered. The title subject is dealt with in papers e.g. on single unit recordings from the visual cortex in cats with induced heterotropias and on photopic spectral sensitivity in emmetropic and normal eyes. The electrophysiological investigation in the presence of cataracts makes up the topic for another important section of the book. In this instance demonstrated that the prognosis of a patient with diabetic retinopathy after a vitrectomy is correlated to the magnitude of the pre-operative photopic response. Other reports deal with electrophysiological patterns in colour vision defects or in peripheral retinal degenerations and anomalies of the optic nerve.

The last part of the book is devoted to various topics such as the effect of stimulus duration on the oscillatory potentials, taurine as an inhibitory transmitter and changes in retinal ganglion cell function during acute elevation of IOP.

All papers are in good English but abstracts are omitted in many cases. The booklet has a natural place in the library of clinics and laboratories with an interest in visual physiology.

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*Rehak S, Arasnov M M & Paterson G D Recent Advances in Glaucoma International Glaucoma Symposium Prague 1976 46 Figs 46 Tables VIII 1977 Cloth DM 75 - US\$ 39.00 Berlin Heidelberg New York Springer Verlag Prague Verlag Avicenum ISBN 3 540 0 944 0*

In 1966 an international glaucoma symposium was held in Prague-organized by the Czechoslovakian Ophthalmological Society in cooperation with All Union Ophthalmological Society USSR. The reports from the meetings are now published in a book comprising a little less than fifty papers.

ut half of these are studies from Eastern Europe and the rest from the western (one Scandinavian) and Japan. Several of the very well known glaucoma contributed to the symposium with papers and discussions. The theoretical part of the symposium concerned the flow of blood and aqueous through the eye and the problem of the outflow of aqueous humour. The clinical part comprised glaucoma surgery Laser treatment conservative treatment of open angle glaucoma the problems concerning screening early diagnosis and hypertension. After each paper there are references and the discussion is held after each topic. There is a subject index and an author index. In the surgical part there is of course a discussion of trabeculectomy and how it is done. The indications and selection of operation of primary glaucoma and the indications of antiglaucomatous operations are stated. A couple of more unusual procedures are goniospasm diathermo trabeculotomy ab externo and the long term results of microdrainage of the anterior chamber using a hydron capillary drain are described. The problems of screening for the early diagnosis of primary glaucoma are discussed once again. General screening is not to be advocated, but the advice is given to each patient who visits the ophthalmologist at least if he is over 50 years of age. This is closely connected with the next item ocular hypertension and how to treat it. The discussion is very informative and helpful in this difficult matter. The new Beta adrenergic and Beta adrenolytic drugs are studied in an Italian study. The strange fact is emphasized that both adrenergic beta receptor agonist and beta receptor antagonist are effective in reducing the intraocular pressure. The effect of different drugs from the two groups are studied. This symposium has covered the most important world wide problems of glaucoma in a satisfactory way. There are good discussions and interesting reports. As a small objection, it must be mentioned that there are quite a few misprints and some translation errors.

*Poul Ager*

*Arrets Zoralia* Diabetic Retinopathy 120 pp 220 Figs Masson Paris Price 150 Fr

The author describes different aspects of diabetic retinopathy such as prevalence individual features major clinical types the natural course medical treatment including photocoagulation and diabetic retinopathy and pregnancy. In the last two chapters the author discusses the results of photocoagulation and vitrectomy in this disease. In his opinion which is in agreement with many other statements in the literature photocoagulation is of great value in many cases especially those arising from proliferative diabetic retinopathy. It is furthermore concluded that vitrectomy which is a difficult operation with many pitfalls should be reserved only for cases where photocoagulation treatment has been ineffective. In other words the indications for vitrectomy as a rule begin at the point where those for photocoagulation end. The book is instructive and can be recommended for practicing ophthalmologists as well as those dealing with the treatment of diabetic retinopathy.

*Hans Walther Larsen*

**Jared M Emery & David Paton** Current concepts in Cataract Surgery. Proceedings of the Fourth Biennial Cataract Surgical Congress. The Ciba Company St Louis 1976 515 pages numerous photographs figures comprehensive subject index (3-4000 references) 8 contributions

The book is a report on the 4th Cataract Surgical Congress in 1975, as organiser and chairman. It is divided into 162 short chapters which are under 9 main sections namely 1) preoperative considerations (incl. anaesthesia instrumentation) 2) intracapsular cataract extraction 3) extracapsular extraction 4) special problems in cataract surgery (pre-existing glaucoma, corneal topography etc) 5) intraocular lenses 6) operative complications 7) postoperative complications 8) correction of aphakia 9) ambulant bilateral or high volume cataract surgery.

The book contains an abundance of useful information on cataract surgery of the anterior segment. It makes light reading due to its unorthodox and straight forward presentation. In spite of the multifarious contributions from different authors the book is quite comprehensible with a vast subject index and extensive use of boldface types in formulating the more general and especially the viewpoints or results. The book represents a stimulating and interesting but not a kaleidoscope of valuable details concerning methods, results, experience and opinions from the frontlines of advanced cataract surgery.

There is hardly any problem or question large or small concerning cataract surgery which is not dealt with in this book. The book is therefore considered as being extremely useful, didactic and stimulating for any eye surgeon who performs cataract surgery.

Eshel G. G. G.

**Harold A Stein and Bernard J Slatt** Fitting Guide for Hard and Soft Contact Lenses. Mosby 1977 Price \$ 24.95 (US)

Contrary to other books on contact lenses with the traditional chapters covering history, anatomy and physiology of contact lenses, this book deals with the problems which arise when fitting contact lenses. The book is suitable not only for the new beginner but also to the more experienced contact lens fitter.

The book comprises four sections together with an appendix.

The first section deals with the terminology, patient selection, and indications and contraindications for contact lenses.

The second section is concerned with soft lenses, the various types including bandage lenses. The fitting procedure, cleaning and disinfection. The third section deals with the hard lenses, the fitting procedure, special problems with keratoconus, astigmatism and aphakia.

The fourth section contains definite guide lines for the prescribing of contact lenses.

The appendix contains a number of tables such as a keratometer conversion table.

The book is well illustrated with many good drawings and pictures. It is very comprehensible. The book is indispensable for the new beginner and contains good advice for the more experienced fitter. The book is to be recommended.

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us Jacobsen (Ed) Handbook of Sensory Physiology vol III Development of Sensory Systems Springer Verlag Berlin Heidelberg New York 469 pp 1978 DM 250 Dkr ca 578 -

book deals with the development of sensory systems and in particular emphasizes aspects of their structure and function which could not be derived from the study fully developed systems. Looking at successive developmental stages of a sensory system permits unique insights which would have been much more difficult if not possible to obtain from studying the adult system.

The work is intended as a reference for researchers in developmental biology, neurophysiology and behavioural sciences. In the field of ophthalmology it cannot be claimed to have a general professional interest but it would seem to be most helpful for those particularly engaged in the study of development aspects of the visual system.

The book contains nine chapters of which only two (chapter 4 and 7) are on the visual system. Chapter 4 by David Ingle describes the development of visual behavior in mammalian vertebrates while chapter 7 by Helmut Hirsch and Audie Leventhal describes functional modification of the developing visual system of mammals. This chapter is an interesting review of experiments on binocular and monocular visual deprivation in an actual field in amblyopia research.

The book belongs to the monumental Handbook of Sensory Physiology which should be known to ophthalmologists and used as a valuable reference work.

Niels Ehlers

bert F Schmidt (Ed) Fundamentals of Sensory Physiology Springer Verlag New York Heidelberg Berlin 986 pp 1978 DM 33.60 Dkr ca 195 -

The aim with this introductory text has been to guide the student in biology and the other natural sciences into the realm of sensory physiology. Where other areas of neurophysiology are involved reference is made to a companion volume Fundamentals of Neurophysiology (1978). The two together present an integrated introduction to the neurosciences.

Besides a general introduction to the subject and a chapter on the neurophysiology of sensory systems the book contains chapters on somatovisceral sensibility, vision, hearing, sense of equilibrium, taste, olfaction, thirst and hunger. Each chapter is brief and may be considered as a presentation of the present day status of the field. References to literature and alternative viewpoints are briefly mentioned.

To an ophthalmologist it can be looked upon in two different ways. One can focus on the chapter on physiology of vision by Grusser and Grusser Cornblith and find an almost too short and concentrated presentation of the subject. Alternatively one can focus on the other chapters and find a readable text well suited to broaden the physiological background knowledge.

Many of the illustrations are excellent and may be found useful by students as well as teachers.

Niels Ehlers

## VARIA

### *International Symposium on Herpetoc Eye Disease*

will be held in Freiburg April 12th-14th 1980 Deadline for the submission of abstracts for the Herpes Symposium will be December 15th 1979

For further inquiries please contact

Dr Rainer Sundmacher Universitätsaugenklinik Albinstr. 11 5300 Freiburg  
Fed Rep Germany

### *IV Symposium - Metabolic Eye Disease*

The Fourth Symposium of the International Society on Metabolic Eye Diseases will be held in Cairo Egypt February 11th to 13th 1980 under the auspices of President Mohammed Anwar El Sadat The Program will include Nutritional Metabolic Eye Disease Metabolic Cataracts and the Role of Metabolism in Ocular Infection

A second session of the Symposium will be held in Jerusalem Israel February 14th to 21st 1980 to deal with Diabetic Retinopathy Mucopolysaccharidoses and Retinal and Corneal Disorders

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*University Eye Department (Chief T. L. Thomassen)  
Rikshospitalet University of Oslo*

## CHOROIDAL FLUORESC EIN ANGIOGRAPHY IN RABBIT

BY

AMUND RINGVOLD and ERLING GRØNVOLD OLSEN

The extreme permeability of the choriocapillaris to sodium fluorescein makes angiography with this tracer unsuitable to show the choroidal vascular bed. In order to prevent this profuse leakage the choroidal circulation is visualized with fundus angiography after injection of fluorescein isothiocyanate (FITC) labelled dextrans of different molecular weight: 20 000, 40 000, 70 000 and 150 000. It is shown that the two first dextrans leak out of the choriocapillaris rather rapidly whereas the last two dextrans remain intravascularly long enough to enable good demonstration of choroidal vessels. The practical application of these findings is discussed and it is concluded that the dextran with molecular weight 70 000 should be preferred for use in humans.

*Key words:* angiography - dextran - fluorescein - rabbit - capillaris - choroidal vessels - permeability

Normal and pathologic retinal circulation as well as abnormal barrier conditions have been extensively studied with fluorescein angiography and in many cases this approach has shed new light on the aetiological factors involved in ophthalmic diseases such as central serous retinopathy, pigment epithelial detachment and others. A similar procedure to visualize the choroidal circulation would certainly also be of great value but so far no method has been developed which can demonstrate the choroidal vascular pattern clearly. Some information concerning this circulation is obtained both from conventional fluorescein angiograms (Hyvarinen et al 1969, Oosterhuis & Boen Tan 1971, Archer & West 1974, Hayreh 1974) and from infrared fundus angiography after indocyanine green injection (Hill & Young 1975, Craandijk & van Beck 1976).

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Flower & Hochheimer 1976) and with choroidal absorption of patent blue V (Sorensen 1977). However the results are impeded by the intervening pigment epithelium and in addition, the free diffusion of fluorescein from the choriocapillaris into the extravascular space from the individual choroidal vessels in all but the very early phase of

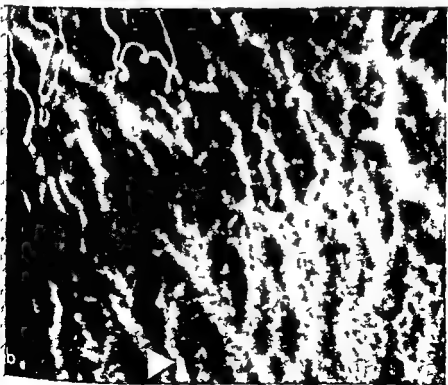
The present study was started as an attempt to overcome these problems. In order to prevent blurring in the angiograms due to leakage of fluorescein from the fenestrated choriocapillaris we used a study of choroidal angiography with fluorescein bound to large dextran particles.

## Material and Methods

This investigation includes two adult rabbits (Dutch and Chinchilla) sedated with 0.15–0.20 ml flumazenium (0.1%) (Hypnorm vet.) intramuscularly about 10 min before angiography. The experiments were performed on the right eye after maximal mydriasis with cyclopentolate (1%) and phenylephrine (10%) drops. The fluorescein was administered either as the conventional 10% sodium fluorescein solution or as a 20% solution of fluorescein isothiocyanate (FITC) conjugated with dextran in Ringer (10 ml). (The dextrans were generously provided from Pharmacia Ltd. Sweden.) The degree of fluorescein substitution of the dextran was one residue per 100–1000 glucose residues (Bellhorn et al. 1971). The FITC solutions (10 ml) therefore gave insufficient vascular filling (unpublished). Both animals underwent angiography successively with particles of four different molecular weights (20 000, 40 000, 70 000 and 150 000) later referred to as FITC 20, FITC 40, FITC 70 and FITC 150 and there was always at least a one week interval between each angiogram on every animal. Osmolarity estimation of the different solutions with an Osmometer gave the following values (mOsm/kg): Ringer 285, FITC 20 301, FITC 40 301, FITC 70 337, FITC 150 335. The dye solutions were injected as rapidly as possible through a marginal ear vein but due to the increase in viscosity of the solutions with increasing dextran molecular weight (Bellhorn et al. 1977) the injection time was considerably prolonged (FITC 150) towards the higher molecular weights. The first angiogram (phase) were taken successively some few minutes after the injection.

Fig. 1

Choroidal angiography of a rabbit (Dutch). a) sodium fluorescein b) FITC 20 c) FITC 40 d) FITC 70 e) FITC 150. One particular choroidal vessel shown in all Figs. 1b–1e.





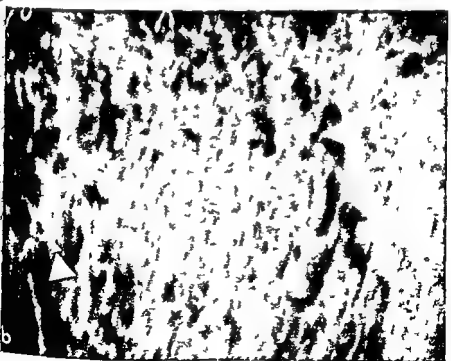
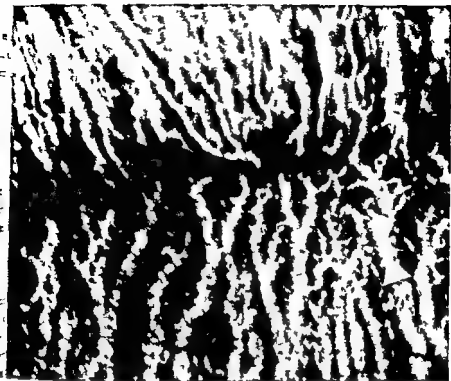
completed whereas the late phase was taken one hour later. Zeiss Camera with Robot Motor Recorder and Nikon wide angle lens. Retinapan 45 were used. The respective equipments were run with Kodak Tri-X film developed in Ilford D11.

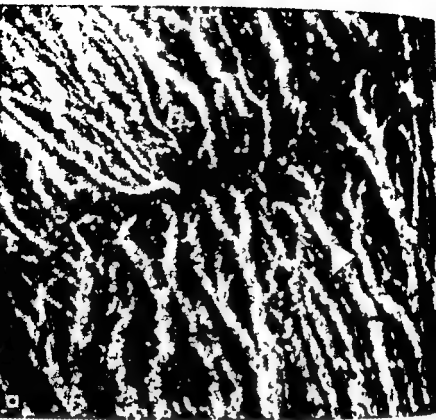
## Results

Fundus angiography after injection of 5 ml sodium fluorescein showed animals the well known sequence of diffuse choroidal fluorescence massive extravasation from the choriocapillaris and distinct retinal vessels (Fig. 1 a). In contrast to this after fluorescein injection choroidal vessels appeared in all early phase and the FITC 20 tracer allows identification of larger vessels only as there is decreasing leakage of the tracer with increasing molecular weight of the dextrans (Figs. 1 b-2-3) but even FITC 150 leaks out in time and some blurring of the vascular pattern one hour after the injection. Since slight haziness of the vessel contours are also present in early phase after FITC 150 injections (Fig. 2 a) one has to base details of the choroidal vascular bed on early angiograms with the FITC 150 tracer. For comparison it was intended to visualize retinal and choroidal vessels in the same field and accordingly most angiograms show the fundus. A part of the fundus just below the papilla is seen in Figs. 1 b-3 b. Arrows in these pictures indicate one particular choroidal vessel from angiograms from Figs. 1 b-3 b. In this region striking zone towards which choroidal vessels from both sides taper off (Figs. 1 b, 3 a) it is illustrated that the larger vessels in this particular parallel divide dichotomously and give off smaller branches that apparently are transformed rather abruptly into the non-vascular meshwork. The coarse grained appearance of the single choroidal vessel (Fig. 3 a) may perhaps represent numerous smaller connections to the meshwork or it may have been brought about by the granular pigment epithelium. The smallest choroidal vessels visualized with FITC 150 are of the same diameter as the retinal vessels in the peripapillary area (Figs. 2 a-3 a) i.e. about 100  $\mu$ m across (Pince 1964). A distinction between arteries and veins seems impossible in the angiograms because of the

Fig. 2

Choroidal angiography of a rabbit (Dutch) FITC 150 early (a) and late (b) phase one hour after injection





creasing intravasal fluorescein concentration had become high enough to make photographs all the vessels were filled with tracer substance. Principally the same findings were obtained in the Dutch (black and white) and the Japanese (brown) rabbits although the choroidal vessels appeared more granular in angiograms from the brown rabbits. It should be stressed that both rabbits were in good condition throughout the experiments and toxic effects were neither noted during the injections nor in the post experimental period.

## Discussion

As seen in the present illustrations the choroidal vessels appear quite clearly after injection of the larger dextran molecules. This indicates that although usually there is some blurring of the choroidal circulation in angiograms due to the intervening pigment epithelium the extravasation of fluorescein seems to be the main reason why ordinary fundus angiography fails to show details of the choroidal vascular bed. However on the whole the angiograms show choroidal vessels more indistinctly in the brown than in the black and white rabbit and whether this reflects real differences in the choroidea or refers to factors in the pigment epithelium cannot be determined from our results. It has been demonstrated in many species that the wall of the choriocapillaris is penetrated by numerous circular fenestration (about 80 nm across) closed by a diaphragm (Rohen 1964; Hogan et al 1971). These capillaries are highly permeable to large molecules such as myoglobin (mol wt 17 000, 4 nm diameter) whereas the still larger molecules albumin and gammaglobulin (7 nm and 11 nm diameter spheres respectively) are drained at a lower rate (Bill 1968). This impaired or controlled leakage of high molecular serum components may refer to permeability properties of the diaphragms. As expected from these data the ability to prevent leakage of fluorescein from the choriocapillaris depends on the molecular size/weight of the dextran particles to which it is conjugated but since even globulins pass out in time in substantial amounts it was not clear what size of dextran particles would be sufficient to prevent the choroidal flush by fundus fluorescein angiography. We therefore used four different dextran tracers (mol wt 20 000, 40 000, 70 000 and 150 000) with the diameters 6.4 nm, 9 nm, 11.8 nm and 14.4 nm respectively (Pharmacia Fine Chemicals technical data bulletin) hoping that leakage of the smaller ones would be slow enough to show choroidal

**Fig 3**  
Choroidal angiography of a rabbit (Dutch) FITC 150 early (a) and late (b) phase (one hour after injection)

vessels at least in the early phase of angiography. It turned out that FITC 40 angiography revealed definite choroidal vessels but unfortunately marked blurring already in the initial phase. This probably results from the prolonged injection time caused by the high viscosity of the rather small dextrans penetrate the choriocapillaris in substantial amount before the intravascular marker concentration is high enough to demonstrate of the choroidal vessels angiographically. According to the visualization of the choroidal vessels was first achieved after FITC 70 and 150. The leakage of the FITC 150 was so slow that the vessels appear rather distinctly even in the late angiograms after the tracer injection.

In conclusion fundus angiography after injection of fluorescent dextrans is a new tool to study not only retinal disease (Rabkin et al.) but also choroidal circulation and pathology in vivo. Since some red cell aggregation occurs after injection of dextrans with higher molecular weight such as FITC 150 (Stalker 1961) it seems that FITC 70 is the most attractive tracer for use in humans. This dextran particle size has been used in Macrodex (Pharmacia) for clinical purpose although not as a fluorescently labelled compound.

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## MACULAR OEDEMA IN THE REATTACHED RETINA

BY

PREBEN GILBERT NIELSEN

A material consisting of 24 consecutive patients has been investigated respectively after a successful retinal detachment operation. Macular oedema was demonstrated by fluorescein angiography in nine patients (37.5%) and was found to be the most frequent cause of poor recovery of visual function. Fluorescein angiography is essential for the diagnosis of macular oedema which may be difficult to demonstrate by other methods. The present investigation indicated that subretinal drainage may be a factor in the development of a post detachment syndrome with macular oedema.

**Key words:** macular oedema - retinal detachment - fluorescein angiography - subretinal drainage

Macular oedema is a potential complication during the postoperative period following ocular surgery. In this respect Cass & Norton (1967) demonstrated using fluorescein angiography the retinovascular origin of macular oedema in the postcataract extraction syndrome.

Macular oedema has long been known to be a postoperative complication following retinal detachment also in phakic patients.

Poor recovery of visual function is a not infrequent occurrence after successful retinal detachment surgery with an apparently uncomplicated postoperative course. A deterioration in visual acuity can occasionally be observed despite satisfactory initial results. Until recently fluorescein angiography of the macula in the reattached retina have only been referred to sporadically.

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literature Cleary & Leaver (1978) published a systematic study of the detached macula using fluorescein angiography. The present investigation was commenced in order to study a material of patients with retinal reattachment using fluorescein angiography. Particular emphasis being attached to the occurrence of macular oedema and possible factors predisposing to this condition.

### Material and Methods

A total of 89 patients were subjected to operation for retinal detachment during a period of two years (1976-78) at the Ophthalmological Clinic of the Odense University Hospital. The following were excluded from the material in order to eliminate abnormalities of complex origin. Those requiring more than one operation for reattachment of the retina, cases with aphakia, detachment preceded to be of traumatic origin, excessive myopia ( $\geq -8D$ ), pre-existing macular disease and cases with opacities in the ocular media. The material was reduced in this manner to 28 patients. Two failed to attend the follow-up examination and two had died. Thus there remained a total of 24 patients who have been examined at intervals varying from 3 to 26 months post-operatively. The visual acuity of each patient was determined. The fundus of each eye was examined by direct and indirect ophthalmoscopy and the condition of the macula evaluated biomicroscopically. Colour photographs were taken and fluorescein angiography carried out on all patients. The duration of the retinal detachment varied from one day to in a single patient three months. The average duration of retinal detachment was 12.8 months and the macula was detached in 14 of the 24 patients. Their ages ranged from 18 years to 78 years with an average age of 63 years.

#### *Method of operation*

All the patients were subjected to a scleral buckling procedure. Local silicone explant was employed in 13 cases. An encircling procedure alone or combined with local explant was used on the remaining patients. In 11 cases cryopexia and diathermia was employed through full thickness sclera. In the remaining 13 cases no cryopexia or diathermia was used. Subretinal drainage was carried out in 22 of the 24 cases. This was estimated to be excessive in seven patients, moderate in 12 and in three patients only that drainage was necessary.



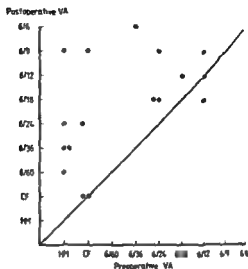


Fig 1

Scattergram showing changes in visual acuity (VA) in 24 eyes after retinal detachment surgery (HM = hand movements (F = counting (in cm))

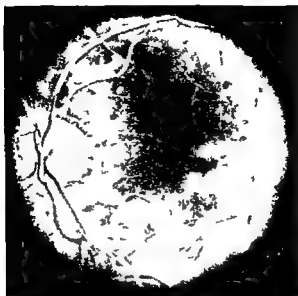
## Results

The changes in visual acuity are shown in Fig 1. The visual acuity was unchanged in three and improved in the remaining 21 (20) at the time of follow up examination and as compared to preoperatively.

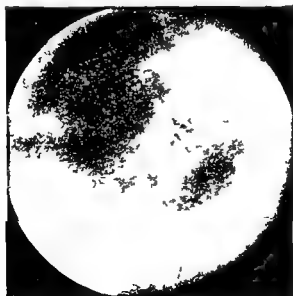
Macular oedema was found in a total of nine patients (35%). The examination revealed macular oedema in five patients only. Fluorescein angiography verified the presence of the condition in all five patients and histopathology demonstrated macular oedema in a further four cases where no signs of this abnormality could be observed during the clinical examination. In six of these nine cases the angiograms showed typical cystoid degeneration with a central dark stellate figure surrounded by fluorescein permeating intraretinal cystoid spaces. In the remaining three patients the oedema was of the amorphous type.

The presence of macular oedema was synonymous with a visual acuity of 6/18 or less with the exception of one patient where the visual acuity was 6/12. Fig 4 shows the visual acuity of the patients with macular oedema compared to those without this complication.

A reduction in visual acuity was observed in four patients at the follow up examination when compared to the visual acuity on discharge.



*Fig 2*  
fluorescein angiogram late phase (11 min) showing macular oedema at three months



*Fig 3*  
fluorescein angiogram late phase (30 min) showing typical cystoid macular oedema at 13 months

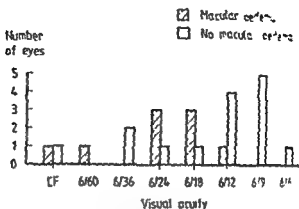


Fig 4

Histogram showing visual acuity postoperatively in nine eyes with and without macular oedema

10 postoperatively) It was possible to demonstrate macular oedema at the follow up examination in three of these four patients who showed a reduction in visual acuity

Macular oedema could be demonstrated in five of the seven eyes with excessive subretinal drainage while with moderate drainage it was observed in four of 12 cases. No oedema at the posterior pole could be seen in any of the five cases where there had been none or only slight drainage

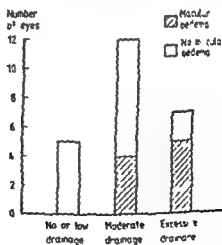


Fig 5

Histogram showing number of eyes with and without macular oedema according to degree of subretinal drainage

## Discussion

Macular oedema has been demonstrated in 35% of the patients in the present investigation by means of fluorescein angiography after an otherwise uncomplicated operation for retinal detachment. An attempt has been made to exclude from the material other possible cases of macular oedema of non detachment origin. In a prospective study of a similar material where fluorescein angiography was systematically carried out Cleary & Leaver (1978) found macular oedema in 17 of 66 cases (25.7%). Bernardczykowa (1975) demonstrated biomicroscopically macular oedema in nine of 30 examined patients (30%). In the present material subretinal drainage was carried out in approximately 92% of the cases. In comparison subretinal drainage was used in only approximately 40% of the cases in the material of Cleary & Leaver (1978). The operative technique employing subretinal drainage in addition to scleral buckling inevitably involves considerable manipulation and formation of an extremely soft eye. Changes in the vitreo retinal interface with vitreous detachment at the posterior pole is a likely consequence and vitreo retinal traction a natural result via the normally existing vitreo macular adherence (Grignolo 1952). The development of a post detachment syndrome with macular oedema can hardly be ascribed to any single factor. In the present investigation subretinal drainage appears to have played a role. An actual comparison of the drainage technique with the non drainage technique has however not been possible in the present study where all but two of the patients were subjected to drainage to a greater or lesser extent. The material has been subdivided according to the estimated extent of the subretinal drainage and this revealed an accumulation of cases with macular oedema in the group with excessive drainage (Fig 5). In the material of Cleary & Leaver (1978) 10 of 21 eyes with drainage showed macular oedema (37%) as compared with seven of 39 eyes without drainage (18%). This difference is statistically non significant with  $0.05 < p < 0.1$  ( $\chi^2$  method). No correlation could be demonstrated between macular oedema and age of the patient, refraction, the duration of the detachment or preoperative macular detachment. There is no evidence in the results indicating that use of cryopexia, diathermia or local explant contra encircling procedures are factors of importance with regard to the development of macular oedema. Regarding the visual prognosis in the nine patients with macular oedema it must be considered poor in at least five cases. In the five cases the duration of the macular oedema was more than one year (observation time 3-36 months). The observation time in the remaining four cases was less than one year and the final visual outcome therefore still uncertain. Cleary & Leaver (1978) found persistent macular oedema in seven out of a total of 17 cases.

*Table 1*  
Macular oedema in relation to subretinal drainage

	+ macular oedema	- macular oedema
<i>Present material</i>		
+ subretinal drainage	9	13
- subretinal drainage	0	1
	9 (30%)	14
<i>Cleary &amp; Leuter (1965)</i>		
+ subretinal drainage	10 (31%)	11
- subretinal drainage	7 (18%)	37
	17 (25%)	48

It has been found in the present investigation that macular oedema was the most frequent cause of poor recovery of visual function. Persistently reduced vision could however not be ascribed to demonstrable macular abnormalities in all of the cases. Two patients with normal macular appearance both clinically and angiographically obtained maximal visual acuity of 6/6 and 6/12 respectively. In these two cases the reduction in visual acuity must presumably be ascribed to failure of retinal receptor regeneration. It is known from experimental studies that retinal detachment is rapidly followed by degeneration of the retinal receptor cells and that reattachment is followed by regeneration (Kroll & Machemer 1969). Previous assumptions of poor receptor regeneration as a relatively frequent cause of severely reduced vision (Chisholm et al 1975, Gundry & Davies 1974) could not be confirmed in the present study where macular abnormalities could be demonstrated in the great majority of patients with severely reduced vision. However incomplete regeneration of the retinal receptors could be considered as the explanation why only one patient obtained a visual acuity of 6/6.

It may be concluded that macular oedema is a frequent complication and must be suspected in cases of poor recovery of visual function after retinal detachment.

t of the retina or where visual acuity suddenly worsens during the post operative period. Fluorescein angiography is an essential part of the diagnosis of macular oedema.

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# VITRECTOMY AFTER INTRACAPSULAR CATARACT EXTRACTION IN DIABETIC SEQUELAE

BY

PEEP ALGVERE

Pars plana vitrectomy was performed on 40 diabetic eyes with opacified vitreous due to preretinal haemorrhages and fibrovascular proliferation. Cataractous lenses were present in all cases and were removed by intracapsular extractions 4-8 weeks prior to vitrectomy. Preoperative visual acuity ranged from light projection to 1/60.

Postoperatively vision improved in 26 (65%) eyes remained unchanged in 5 (12.5%) and deteriorated in 9 (22.5%) during a follow up time of 1-15 months (mean 8 months). In 13 (47.5%) eyes a visual acuity range from 0.1-0.9 was achieved. In 31 eyes that improved or remained unchanged iris neovascularization developed in 2 (6%) and longstanding increase of intraocular pressure in 9 (29%). Visual failures were mainly due to incomplete removal of preretinal proliferations and retinal detachment and neovascular glaucoma developed in 5 of the 9 eyes. In 30 (75%) eyes the anatomical status at the vitreo-retinal junction improved considerably when the preretinal tissue causing retinal traction was excised and the proliferative disease reverted into a background retinopathy.

**Key words:** vitrectomy pars plana - diabetes mellitus - cataract extraction - visual improvement - complications - retinal detachment

Since the introduction of special instruments (Machemer et al 1967, Mola 1970, Douvas 1975, O'Malley & Heintz 1975 and others) vitrectomy via pars plana has rapidly become an accepted surgical procedure. The most common indications for vitrectomy so far have been the diabetic sequelae associated with vitreous opacification and preretinal fibrovascular proliferations. In such

There is a high incidence of lens opacities which spontaneously or following vitrectomy often progress to dense cataracts requiring lens removal (Faulborn and 1978). Accordingly lensectomy *via* pars plana in the same session as vitrectomy has been widely practiced (Machemer 1975). However hard nuclei are difficult to remove with any of the vitrectomy instruments now available making ultrasonic fragmentation of the lens a necessity. Although optical clarity is achieved the lens tissue cannot be entirely removed by lensectomy and the lenticular remnants tend to aggravate the postoperative inflammation.

It has been suggested that (even in connection with vitrectomy) sclerotic lenses be extracted intracapsularly with consequent complete removal of all lens tissue (Tolentino et al 1977). However performed at the same time as vitrectomy intracapsular lens extraction may be a more traumatizing procedure than pars plana lensectomy.

In the present study vitrectomy was performed on diabetic eyes with long standing vitreous haemorrhages and periretinal fibrovascular proliferations associated with cataractous lenses. The lenses were removed by intracapsular extractions. In an attempt to minimize the surgical trauma a two step procedure was adopted the vitrectomy was performed 4-8 weeks after the cataract extraction and not before the inflammation following lens surgery had subsided.

## Materials and Methods

Between April 1977 and March 1978 cataract extractions and pars plana vitrectomies were performed on 40 patients (18 males 22 females) ranging in age from 30-72 years. The patients had been known diabetics for 7 to 47 years (mean 29 years). All 40 eyes had proliferative diabetic retinopathies and vitreous haemorrhages which lead to longstanding vitreous opacification. Loss of ambulatory vision was recorded between 1 and 12 years (mean 3.1 years) prior to operation.

Routine ophthalmologic examination included biomicroscopy with the 3 mirror lens and ultrasonography using A scan and B scan according to Coleman (Algvere et al 1978). Vitreous membranes and preretinal or prepapillary proliferations were observed in all eyes. Localized retinal detachments were present in 13 eyes (33% of the cases).

The following criteria were used for selecting patients. Visual acuity ranged from perception and accurate projection of light to finger counting at 1 m (1/60). Iris neovascularization was considered a contraindication for vitrectomy. Patients with dilated iridial vessels but without glaucoma were not refused.



**surgery** Large retinal detachments (covering at least one quadrant of the fundus) associated with preretinal proliferations were not operated on. Electrophysiologic examinations were done in selected cases. Electroretinograms (ERGs) were recorded in response to strong light stimuli (6 log units above the dark-adapted b wave threshold). An eye with an extinguished ERG was considered to have a poor postoperative visual prognosis and was not vitrectomized. Visually evoked response (VER) was elicited by flickering light of 15 Hz. Positive results indicating macular function definitely encouraged surgery in dubious cases.

**Extraction of the opacified lens** In this series all eyes had lens opacities or scleritis or dense cataracts respectively. An intracapsular cataract extraction was performed using a standard procedure: a corneo scleral (limbal) incision about 160° under a conjunctival flap, preplaced corneo scleral sutures, a serotyped iridectomy, cryoextraction of the lens. Enzymatic zonulolysis was used for patients under 40 years of age. The corneo scleral incision was closed with 10-12 interrupted 8-0 silk sutures. Prophylactic cryo treatment was applied over the pars plana and ora serrata in the region where the sclerotomy for later vitrectomy was planned.

**Vitrectomy** A lotus vitreous stripper and endodiathermy unit were used (O Instruments, St. Gallen, Switzerland). Both instruments are equipped with fiber optic sleeves for intraocular illumination and can be inserted into the vitreous through the same sclerotomy, since their outer diameters are identical.

Under a conjunctival flap, a sclerotomy was made 4-5 mm posterior and parallel to the limbus, usually in the upper temporal quadrant. Weak diathermy was applied to the pars plana in the sclerotomy bed. A perforation mark was placed inside an infusion cannula, which was inserted through the pars plana into the vitreous. The cannula was sutured to the sclerotomy edges. The cannula was withdrawn and an infusion of Ringer solution connected to the cannula (and vitreous compartment). The intraocular pressure was then determined by the height of the infusion column above the eye.

Surgery was performed under a motorized Zeiss (OPMI 6) microscope equipped with X-Y coupling (Zeiss, Oberkochen, W. Germany). A thin and light contact lens was placed loosely on the cornea; abrasion of the corneal epithelium was hardly ever necessary.

The aim of surgery was an almost complete removal of the vitreous, leaving only the vitreous base and a thin cortical layer (when that layer was attached to the retina). The fibrovascular proliferations were cut as close to the retina and optic disc as possible. The resection was very often combined with endodiathermy of preretinal vessels.

ologic examination of the material obtained from the vitreous space was carried out in 12 cases. The aspirated suspension was centrifugated, the precipitated material fixed in formaline, mounted in paraffin, sectioned and stained with haematoxyline eosine or van Gieson dye.

Follow up examinations were performed at regular intervals (monthly for the first 3 months) and the follow up time ranged from 5-18 months (mean 8 months) comprising all patients in the study.

## Results

Phacolytic intracapsular cataract extraction was well tolerated by the diseased eyes. The postoperative inflammation as a rule subsided within four weeks. An unintended extracapsular operation occurred in four eyes leading to a considerably more severe but still transient postoperative inflammation. During



*Fig 1*

Micrograph of proliferating tissue excised from the vitreous space showing fibrovascular proliferations with newly formed vessels and van Gieson staining collagen (original magnification 500x)

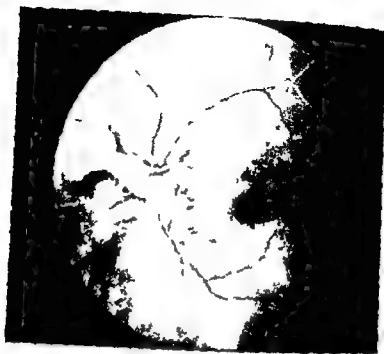


Fig 2

Fundus photograph 14 months after vitrectomy and resection of fibrovascular proliferations of 63 year old male who had been socially blind for 3 years. Duration of disease 45 years. There is a remaining stalk of fibrous tissue in front of the optic disc. Postoperative visual acuity 0.7 (aphakic correction)

the first postoperative week anterior chamber haemorrhage was reabsorbed in several eyes. Large postoperative haemorrhages occurred in five eyes, two of which had very advanced proliferations in the vitreous space and later showed a poor visual prognosis.

The opacified vitreous was successfully removed from the posterior pole of the fundus. The excision of preretinal membranes and fibrovascular proliferations was much more difficult since they were firmly attached to the retina or optic nerve head. Histologic examination of the material removed from the vitreous space showed various blood degradation products such as red cells and fibrin and proliferating fibrovascular tissue. Fibrous tissue was seen surrounding the newly formed vessels and contained van Gieson-stained collagen (fig 1).

The preretinal tissue was successfully excised from 30 eyes and the optical transparency of the vitreous space restored (Fig 2). The anatomical situation at the vitreo-retinal junction was considerably improved when the post-

nal traction was eliminated (Figs 3 and 4) Eyes in which such traction was not eliminated at surgery and vitreo retinal attachment persisted later developed severe complications (see Visual failures)

The recovery of vision occurred mainly during the first two months after vitrectomy but minor improvement was observed up to six months At follow up examinations (on the average eight months after vitrectomy) an improvement of vision was present in 26 (65%) eyes A visual acuity ranging from 0.9 was achieved in 19 (48%) operated eyes (Table 1) Seven eyes gained no visual increase (finger counting 2-5 m) these patients enjoyed some blurry vision that facilitated their social but not their professional activities

Although the optical transparency was restored in most eyes normal visual acuities were not achieved Various sequelae of retinopathy were present and especially macular degenerations were commonly seen after vitrectomy in many cases these were associated with thin epiretinal proliferations that could be excised In the majority of eyes occlusions of retinal vessels were



*Fig 3*

Fundus photograph 6 months after vitrectomy showing persisting vitreo retinal traction leading to a retinal fold which progressed to a detachment (arrows) Male aged 35 years socially blind for 2 years Postoperative visual acuity 0.9 (aphakic correction)



Fig 2

Fundus photograph 14 months after vitrectomy and resection of fibrovascular proliferations of 6, year old male who had been socially blind for 3 years. Duration of disease 45 years. There is a remaining stalk of fibrous tissue in front of the optic disc. Postoperative visual acuity 0.7 (aphakic correction)

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*Table II*  
Primary causes of visual failure (9 eyes)

	Number of eyes
Incomplete removal of fibrovascular proliferations	3
Persisting retinal detachment	4
Profuse postoperative haemorrhage	1
Neovascular glaucoma	1

ountered and circulatory insufficiency was probably the major cause of the unsatisfactory macular function

*Visual failures* In nine eyes a permanent decline of vision and a general deterioration of the ocular disease occurred following vitrectomy (Table II). These cases had extensive preretinal fibrovascular proliferations which were difficult to remove. In three eyes only part of the huge vascular network in the vitreous space could be resected and traumatized fibrovascular tissue had to be left in the vitreous. Postoperatively retinal detachment and neovascular glaucoma developed.

In five eyes retinal detachments were present at surgery, three of which showed retinal breaks in the posterior pole. In one eye an iatrogenous retinal hole was produced when excision of a traction membrane was attempted. Scleral buckling was performed in two eyes but was technically difficult and remained unsuccessful. Since the retinal breaks were located close to the optic disc(s) in areas of fibrovascular membranes that could not be removed, scleral buckling was abandoned in the remainder of the eyes.

One eye developed a profuse postoperative haemorrhage that did not resorb. Vitreous lavage was performed but blood soon refilled the vitreous space and retinal detachment ensued.

Persisting retinal detachment was the major cause of visual failure after vitrectomy and was often associated with glaucoma and iris neovascularization (Table III).

*Increased intraocular pressure (IOP)* A transient increase of IOP (30 mmHg or more) was common during the first postoperative weeks and was mainly of erythroclastic origin. A longlasting increase of IOP (for two months or more)

Table III

Complications observed after vitrectomy in various visual acuity groups of eyes

Visual acuity	Number of eyes	Petinal detachment	Glaucoma	Iris neovascular	Ph <sup>1</sup> 12 bulb
Amaturosis - light percept	9	8 (89%)	5 (56%)	5 (56%)	4 (44%)
Light projection - 1/60	5	1	2	1	
2/60-5/60	7		3 { (43%)		{ (6%)
0.1-0.9	19		4 {	1*	

\* extracapsular lens extraction

was seen in 14 of 40 eyes but in only 9 of the 31 eyes that either improved or remained at the preoperative visual acuity following vitrectomy (Table II). These eyes required medication to normalize the IOP. It must be pointed out, however, that the non vitrectomized fellow eyes of four of these patients had a long history of glaucoma.

**Iris neovascularization** Biomicroscopy (magnification 26 x) and gonioscopy disclosed a transient postoperative dilatation of iris vessels in several eyes particularly in those with new intraocular haemorrhages. This phenomenon was quite marked in eyes that showed iris vasodilatation preoperatively. Postoperative development of iris neovascularization was seen in 7 of 40 eyes in only 2 of 31 eyes without retinal detachment and was associated with recurrent intraocular haemorrhages and long-lasting increase of IOP (Table

## Discussion

The prerequisite for a good surgical result is a technically satisfactory vitrectomy that enables adequate removal of the opacified vitreous and the proliferating fibrovascular proliferations. The two major goals of vitrectomy can thus be fulfilled: the restoration of the optical transparency of the vitreous space and the elimination of vitreo-retinal traction.

These goals can only be achieved if the visualization of the vitreous is satisfactory during surgery. In the present series all eyes showed no opacities or cataracts and therefore a two step procedure was adopted.

ng an intracapsular lens extraction and — 4 to 8 weeks later — a vitrectomy  
vitrectomy and especially the excision of preretinal proliferations is  
mplished much easier in aphakic eyes with sector shaped iridectomies than  
akic ones. In phakic eyes visualization of the operation field may be  
red by such factors as lens opacities, incompletely dilated pupil (e.g. with  
synechiae) or blood in the vitreous space. In addition the two step

re enabled cryo treatment of the pars plana and ora serrata during the  
operation stabilizing the tissues around the sclerotomy for the later  
ctomy. Accordingly no retinal dialysis was observed in this series of  
ations.

asoproliferative substances are assumed to be released from a hypoxic  
ia (Ashton 1961) and may cause iris neovascularization. These agents must  
use into the anterior segment and such diffusion is facilitated when barriers  
as the lens and vitreous are removed. It can thus be argued that following  
ep wise removal of these barriers the vasoproliferative substances probably  
h the anterior segment gradually. On the other hand if the lens is clear  
u h to be left in the eye anterior uveitis, glaucoma and iris rubeosis are  
likely to be induced by the vitrectomy.

n the present series there was an improvement of vision in 65 % of the  
s, and in 48 % a visual acuity of 0.1 or more was achieved. Following  
rectomy for diabetic vitreous complications Mandelcorn et al (1976) found  
overall success rate of 53 % and 43 % of the eyes gained 6/60 or more  
visual acuity. Their recent report on 5 year follow up examinations con-  
med that these surgical results were quite stable and longlasting (Blankenship  
Machemer 1978). Peyman et al (1976) reported an improvement of visual  
uity in 71 % of eyes operated on at least six months after the onset of  
reous haemorrhage. Michels (1978) observed visual improvement in 65 %  
the eyes and in 46 % of final vision of 20/200 or better was achieved.

It is quite obvious that the rate of visual success is dependent on the extent  
preretinal proliferations and the presence or absence of retinal detachment  
operatively. Dense vitreous haemorrhages without fibrovascular prolifera-  
ons generally have a good prognosis following vitrectomy.

In the pre ent series fibrovascular proliferations were found in all eyes  
nce excised such proliferations were permanently eliminated and growth  
f new tissue into the vitreous space never occurred. This is a remarkable  
henomenon showing the value of vitrectomy in diabetic proliferative retino-  
athy. The present study confirms similar observations made by others (Michels  
98 Blankenship & Machemer 1978). The vitrectomy thus changes a retino-  
athy with preretinal membranes and fibrovascular proliferations into a back-  
round retinopathy. The progressive course of the vitreo retinal traction is



interrupted. Thus complications of retinal traction such as detachment and degeneration of the retina and haemorrhages from vitreous retinal and retinal vessels are alleviated or prevented. In addition the toxic blood degradation products are removed from the eye. If necessary the haemorrhagic retinopathy can then be photocoagulated.

Vitreous haemorrhages do occur following vitrectomy. Postoperatively 22 patients reported transient blurring of vision which was caused by recurrent haemorrhages. However in all eyes without retinal detachment (except those with profuse haemorrhages) vitreous blood was spontaneously resorbed within a week leaving no opacities. This favourable course can probably be explained by the fact that in aphakic eyes vitreous blood easily gains access to the anterior chamber and red cells pass out through the chamber angle. In our experiments intravitreally injected red cells did gain access to the anterior chamber in aphakic but not in phakic eyes (Algvere & Bill 1979).

The surgical failures in the present series were mainly due to incomplete removal of preretinal proliferations and to retinal detachments. As previously reported (Algvere 1978) vitreous retinal traction bands persisting after vitrectomy will contract weeks or months later and lead to retinal detachment. These show the most severe complications including iris rubeosis and neovascular glaucoma.

In this series the overall incidence of postoperative iris neovascularization was 18%. However if the eyes lost to retinal detachment are subtracted from this figure only 6% developed iris neovascularization. Iris rubeosis following vitrectomy was reported to occur in 26% (Mandelcorn et al 1976) to 40% in eyes (Michels 1978) most of which had undergone a pars plana iridectomy at the same session as vitrectomy. The two step procedure with extracapsular cataract removal and later vitrectomy seems to be preferable in respect to the postoperative development of iris rubeosis.

Increased intraocular pressure following vitrectomy in diabetics is common and was reported to occur in 9% (Gitter & Cohen 1976) to 49% of eyes (Weinberg et al 1976). In the present series elevation of IOP for at least two months was encountered in 35% of eyes but if those lost to retinal detachment are excluded the figure drops to 29%. Neovascular glaucoma developed in the group of surgical failures. In the remainder of the cases neovascular glaucoma was present in association with persistent retinal detachment (one eye) and following extracapsular cataract extraction (one eye). Usually both types of glaucoma were seen (erythroclastic or haemolytic corticosteroid-induced simplex glaucoma) some of which would probably have developed without vitrectomy since they were present in the non operated fellow eyes.

## Addendum

follow up periods of 6-26 months (median 16 months) 55 eyes operated with these methods showed the following results The visual acuity had improved in 11(20%) eyes remained unchanged in 8(14.5%) and improved (60.5%) In 29(53%) eyes a visual acuity ranging from 0.1 to 0.9 was achieved In the groups of improved or unchanged vision glaucoma was seen in 3(5%) eyes and iris neovascularization in 2(5%) eyes  
The results were generally stable and lasted during the longer follow up periods

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## Material and Methods

The material comprised 92 patients ranging from 40 to 91 years of age operated for senile cataract. The assessment was based on a double-blind investigation. All patients had premedication consisting of 10 mg d.i. (Valium®) i.m. about one hour preoperatively.

The retrobulbar injection of 5 ml of the drug was made through the conjunctiva with a 3 cm long blunt needle.

Lidocaine 2% with adrenaline (12.5 µg/ml) (epinephrine) was given to 33 patients, mean age 70 years. Lidocaine 1% was given to 27 patients, mean age 75 years. Etidocaine 1% with adrenaline (5 µg/ml) (epinephrine) was given to 24 patients, mean age 72 years.

In all groups hyaluronidase (Penetrase®) was added to the anaesthetic at a concentration of 200 IU/ml.

For facial nerve block 5 ml of the respective anaesthetic preparation, with adrenaline, was injected in the preauricular area and along the facial nerve branch towards the orbicularis oculi. Various doctors performed the anaesthesia, but the technique has been identical.

Average interval between the injection of the anaesthetic and the commencement of the operation was 18 min (12–20 min) in all the three groups. During this time the intraocular pressure was reduced by massage to 14 (Schiotz) or lower.

Table 1  
Effect of lidocaine and etidocaine  
The need for additional injection in brackets

	Lidocaine 2% adrenaline	Etidocaine 1%	Etidocaine 1% adrenaline
Satisfactory akinesia and anaesthesia	43	5	16
Unsatisfactory akinesia of the eye lids	1 (1)	0 (3)	0
Unsatisfactory akinesia of the eye bulb	1 (1)	0	5 (1)
Pain during operation	1	3	3 (1)
Total no. pat.	46 (2)	27 (3)	24 (1)

## Results

Table I comments concerning the effect of the anaesthesia and the need for injection of the anaesthetic agents are summarized. The table shows that unsatisfactory akinesia of the eye lids and the eye bulb unsatisfactory anaesthesia was observed more frequently in the two etidocaine groups. The difference is statistically significant ( $P < 0.01$ ). Extra injection of the anaesthetic preparation was necessary in 2 of the 46 patients (4.4%) in the lidocaine group compared to 9 of the 46 patients in the etidocaine groups (19.6%) ( $P = 0.02$ ). During the postoperative phase pain relieving tablets were given to 14 patients (30.4%) in the lidocaine group compared to only 6 patients (13.0%) in the etidocaine group. The difference is on the border of statistical significance (0.05). The need for pain relieving drugs occurred on average 324 min after injection in the lidocaine group compared to 400 min in the etidocaine group. There were no retrobulbar haemorrhages, allergic reactions or local or general toxic effects registered in any of the patients.

## Comments

Due to the vasoconstrictory effect of adrenaline there have been misgivings concerning the use of this agent in an anaesthetic preparation to be used retrobulbar, mainly because of the danger of optic nerve ischemia (Thorburn et al 1966; Harven 1978). Especially in the operative treatment of glaucoma with retrobulbar optic nerve damage one ought to consider omitting adrenaline in retrobulbar anaesthesia. The possibility of the general cardiovascular effect of adrenaline must be taken seriously in those patients with cardiac disease such as coronary sclerosis and arrhythmia. While some local anaesthetics including bupivacaine contain adrenaline at a low concentration of  $\mu\text{g/ml}$ , lidocaine does not contain adrenaline. With the retrobulbar anaesthesia and the facial nerve block and the interval between the injection and operation used in this study lidocaine 2% gave a better effect than etidocaine 1%. The necessity for analgesic drugs during the postoperative phase indicated a more protracted action of etidocaine than of lidocaine. These results may be explained by a more rapid onset with lidocaine than with etidocaine. If this was the case the difference in effect might have been negligible if the interval between the injection and the operation had been of a longer duration. However, one of the main requirements of a

good local anaesthetic preparation is satisfactory anaesthesia and analgesia which takes effect within the normal interval between the injection and the commencement of the operation

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# IMPROVED RADIOLOGICAL TECHNIQUE FOR EVALUATING THE LACRIMAL PATHWAYS WITH SPECIAL EMPHASIS ON FUNCTIONAL DISORDERS

BY

ARRIGO MONTANARA, PIETRO CATALINO and MASSIMO GUALDI

A radiological study of the lacrimal drainage apparatus was made in 50 patients with temporary or permanent epiphora. The authors state that the present radiologic technique (macrography serigraphy subtraction) allows the detection on the various lacrimal levels of not only organic lesions but also of functional disorders. They assert the definite superiority of dacryocystography over the other clinical and radiological investigations (scintigraphy roentgen cinematography) in identifying the site and the nature of the various organic lesions in evaluating and interpreting the functional alterations as well as following and judging the effectiveness of treatments.

*Key words:* Lacrimal drainage apparatus - Radiological features of tear flow disorders - Macrodacryocystography - Lacrimal Passages

Abnormalities of lacrimal function have been given different names by the various authors: functional blocks (Demorest & Milder 1955), physiological dysfunctions (Hurwitz & Welham 1975), partial functional obstructions (Montanara et al 1979a), functional disorders (Montanara et al 1979b). All of these conditions share the characteristic that the anatomic patency of the lacrimal passages is not associated with functional patency. To our knowledge this subject has not been systematically discussed from a radiologic viewpoint and

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no adequate review has been made of its various aspects and of the diagnostic material obtainable in such cases. The purpose of this paper is to provide a review and discussion of the subject.

**Clinical Tests** Lavage and incannulation, the various taste (saline, sucrose) and colour tests (Lapsius 1957, Vergez 1961, Jones 1967, Horn 1967, Jones 1968, Murube del Castillo 1973, Hurwitz & Welham 1975) (properly called the Jones fluorescein dye tests), lacrimal electromanometry (Callahan et al. 1965) while under certain circumstances prove to be of considerable diagnostic value, do yield results that are not always consistent or in agreement with each other. Such tests may simply demonstrate an anatomical patency but do not allow definite judgement of the functional integrity of the system (Zappia & Milder 1972). Moreover these methods do not demonstrate the absence (Hurwitz & Welham 1975) or pinpoint the precise location of abnormality.

**Radiological investigations** Lacrimal microscintigraphy (Rossomondo et al. 1972, Carlton et al. 1973, Chaudhuri et al. 1974, Sorensen & Tazeghi 1975) and quantitative scintigraphy (Hurwitz et al. 1975) allow an excellent evaluation of lacrimal function and from this viewpoint are superior to the clinical and radiological methods for evaluating the dynamic of tear flow. However for assessing lacrimal excretory function in the clinical field, however, the evidence thus obtained does not give reliable information concerning the nature of the condition studied nor a determination of the precise location of stenosis and surgical indications are therefore uncertain. These tests, although superb for physiology, are still not as precise as dacryocystography in detecting anatomical abnormalities. Scintigraphy is therefore to be recommended in opinion as complementary to macrodacryocystography which in addition should be performed for the purposes of diagnostic confirmation.

The interest of dacryocinematography (Street & Howell 1961, Trotter & Potter 1970) is limited mainly to the area of scientific research since it can help to elucidate the phases of excretion through an analysis of the various mechanisms acting during tear drainage that are still under discussion (ocular gravity attraction, blinking of the eyelids, gravity force, nasal aspiration). For more roentgencinematography also requires for diagnostic confirmation to follow-up dacryocystography since it provides very limited morphological information due to the fact that the canaliculi are not clearly visible because of their small size. Also the images of the sac and of the lacrimal ducts appear to be lacking in detail due to factors that are inherent in the method itself.



*Fig 1*

lacrimal seriography show a constriction of the internal tract of the right hand com on canaliculus due to the presence of a mucous membrane (arrows) the filling defect always present in the various seriograms. The passage of the contrast medium into the lacrimal sac is unimpeded the sac appears moderately ectatic due to inflammation. Another functional constriction exists in the lower tract of the sac (arrow) only an overall analysis of the various seriograms provides a visual impression of the difficulty of the distension of the lacrimal walls which retards the drainage.

To the left obstruction of the sac



*Dacryocystography* (DCG or serial dacryocystography or macrodacryocystography are synonymous) This technique in our opinion, allows the complete evaluation of lacrimal drainage. In the *canaliculi* exclusive functional changes are uncommon whereas these are more frequent before the internal opening of the common canaliculus. Epiphora and functional disorders under such circumstances may be due to a small mucous membrane (Welham & Henderson 1973) located in the common canaliculus, in the middle of the case (Rycroft 1960) in the inner part (Fig 1) usually because of inflammation of the sac. The precise location of the different alterations may be left undetermined if the examination is conducted without subconjunctival (Keast Butler et al 1973).

In the *sac and lacrimo nasal duct* the delayed drainage is most frequent of functional origin. In this area the promoting factors are the malformations

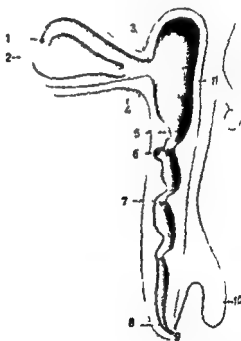


Fig 9

Distribution of the various valve like formations (actually thickenings of the mucous membrane) described in the lacrimal passages: 1) Bochdalek's valve 2) Falz's valve 3) Rosenmüller's valve 4) Mayer's sinus 5) Beraud-Krause's valve 6) Arlt's valve 7) Taillefer's valve 8) Hasner-Cruveilhier's valve 9) lower orifice of the lacrimal duct 10) lower nasal horn 11) insertion of the palpebral ligament.

simple variants of the bony structures (unguis ethmoidal cells nasal fossa) more frequently the drainage defects are due to an inflammatory process of the lacrimal or nasal mucosa or to a simple mucous hypertrophy perhaps of allergic origin. It should be mentioned here that the mucous membrane (called valves) which lines the sac and the duct causes dilatation and constrictions variously described by the authors after whom the different valve formations are named (Fig 2). These should actually be regarded as simple thickenings of the mucosa since neither anatomic preparation nor radiological investigations have confirmed the presence of any such valves.

## Material and Methods

In view of progress in radiological technique (Campbell 1964 Iba & Hanafee 1968 and Lloyd 1975 Montanara et al 1979) it is not possible to detect the presence not only of organic lesions but also of functional disorders of the lacrimal drainage system. To demonstrate such alterations the investigations of choice should be dacryocystography performed under the following conditions:

Bilateral introduction of the contrast medium while the films are obtained

Seriography

Direct enlargement (focal spot of small size not more than 0.3 mm)

Image subtraction

The use of a catheter or needle connected to a catheter protects the operator by enabling him to keep at an adequate distance from the radiation source; the dose received by patient is relatively low with the technique used (80 kV 15 mAs). We have no information in literature that any dosimetric researches on the amount of radiation received by the cornea and the lens during the DCG have been performed. With the technique we use the bulb is affected by a comparatively low amount of radiation certainly not superior to that absorbed during a conventional ray examination of the skull. This in fact needs an almost identical number of mAs and a higher value of mAs in comparison with the DCG with this last technique we make a direct magnification and do not use the radiographic grid. In this way the DCG does not provoke a particular risk for the patient. The problem of the dose maybe of concern to the radiologist particularly if he is not equipped with an automatic skull seriography and an automatic injector. This is why we advise both local lead protections applied on the skull table and use of a catheter which connected to the lacrimal needle allows the operator to stand comparatively far enough away from the x ray tube.

Fifty patients with lacrimal problems (temporary or permanent epiphora) have been radiologically studied by means of macrodacryocystography. In 40 subjects the physical examination syringing of the lacrimal passages and the taste and colour tests were negative; ten patients presented clinical findings of unilateral stenosis.

The contrast medium used was the ultrafluid Lipiodol, advisable because it is homogenous, non irritant, non toxic, is eventually absorbed if not discharged and does not produce a bitter taste. Also it will not form a powdery deposit on the lids.

with subsequent burning as may happen when using water soluble contrast media. The ultrafluid Lipiodol's viscosity (95 centipoises at 31°C) is higher than tears (2916 centipoises Hurwitz & Welham 1975). The water soluble media used for a lower viscosity: Conray 4 centipoises at 31°C Angiografin in 60% solution 10 centipoises at 37°C which is within the normal range for tears. Using the water soluble solution diluted by tears it was not easy to demonstrate radiologically the material in the pharynx thus we prefer to use ultrafluid Lipiodol as functional medium of the kind. There were no adverse reactions to this contrast medium.

All examinations were performed in the antero posterior position. The patient is placed supine on the skull table and some drops of local anaesthetic are instilled in the conjunctival sac. Lloyd's technique is based on a 1<sup>st</sup> inch intravenous cannula bilaterally inserted into the canaliculus we prefer to place a lacrimal needle in the canaliculus (lower or upper) and connect it with the catheter. Two or three ml of ultrafluid Lipiodol are injected in each lacrimal ductus. In order to obtain optimal demonstration of the duct system it is essential not to advance the tip needle more than 3 to 4 mm into the canaliculus (Fig 3). It is the usual practice to carry out bilateral introduction simultaneously in this way useful information can be obtained by comparison with the opposite side.

Immediately following the scout film (performed prior to the injection for identification purposes) two sequential radiograms are taken during the introduction of the contrast medium. In patients with lacrimal physiological dysfunctions we found the films taken 30 sec and 13 and 5 min after instilling Lipiodol gave the most useful information.

Geometrical enlargement of the radiographic image is obtained by placing the beam drawn serial changer some distance from the patients head. With the use of a collimator and a head fixation device subtraction studies may easily be achieved.

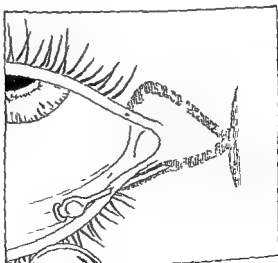


Fig 3  
Correct position of the needle into the canaliculus

# Results

Of 20 patients (100 lacrimal systems examined) we classed 10 of these as anatomically normal. Another 10 subjects presented a complete or incomplete lateral stenosis located at the level of the common canaliculus (2 cases) or at the internal opening of the common canaliculus (8 cases).

The lacrimal systems with purely functional abnormalities and with indications for radiological features were therefore 10. The final results are shown in Table I.

To evaluate Lipiodol's drainage time it is evidently important to know the time in normal subjects. In researches performed by one of us (Monnier 1949) concerning 80 lacrimal systems (40 subjects who showed no anatomical or functional lacrimal alterations and were studied both with ultra-radiopaque Lipiodol and water soluble contrast media) we found that the drainage of the lacrimal system begins a few seconds after Lipiodol's introduction and emptying into the nasal fossa occurs within 6-15 sec (10 sec as a mean) as shown in Table II.

In the normal subjects studied with water soluble contrast media (Conray

Table I

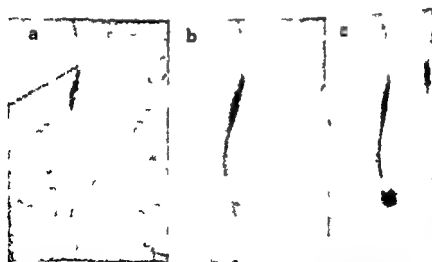
Radiological features	Lacrimal systems examined
Normal lacrimal drainage apparatuses	20
Anatomical alterations (unilateral stenosis or obstruction) The opposite side presented constantly functional disorders	10
a) sac deformed or deviated (unilateral)	11
b) disproportion between the relative diameters (unilateral)	12
Functional disorders	
c) alternating dilated and constricted tracts (unilateral)	15
d) skipping of a tract (unilateral)	7
e) prolonged drainage time (unilateral)	25
Total	100

*Table II*  
Flow time of the contrast media in normal subjects

Normal lacrimal systems examined	Angiografin Conray 35 %	ultrafluid lipodol
30	40	40
initial drainage time (minimum maximum)	Mn = 2 seconds Mx = 4 seconds	Mn 6 seconds Mx 12 seconds
mean drainage time	2.2 seconds	10 seconds

or Angiografin diluted at 35-40 %) the drainage time was found to be shorter two sec after introduction the contrast medium was already present in the nasal fossa (Table II and Fig 4)

The persistence of the contrast medium in the sac and in the duct is completely opacified after more than 30 sec from the introduction; with any passage into the nasal fossa in our opinion should be regarded as a sign of functional disorders. It is not possible to standardize the sequence of various radiograms as a general rule we recommend carrying out a series of (2 films in addition to direct examination) in the first 10 sec during the m-



*Fig 4*

A normal case studied by automatic seriography (two images/sec) and subtraction. At the end of the 2nd sec the contrast medium (water soluble) opacified the nasal fossa.

of the ultrafluid Lipiodol and subsequent control films at 1-3-5 min. The value of 30 sec. which was chosen as a parameter to determine the discharge as normal represents the minimal value required to suspect the presence of biliary disorders. More reliable elements of judgement are offered by successive serigraphical controls eventually performed after 1-3-5 min following the introduction of the contrast medium.



*Fig. 5*

serigraphical serigraphy. On the left side the sac appears off axis (arrow) and slightly angled outwards (see comparison with the healthy side). The contrast medium stops at the level of the lower segment of the sac and later of the lower tract of the duct.

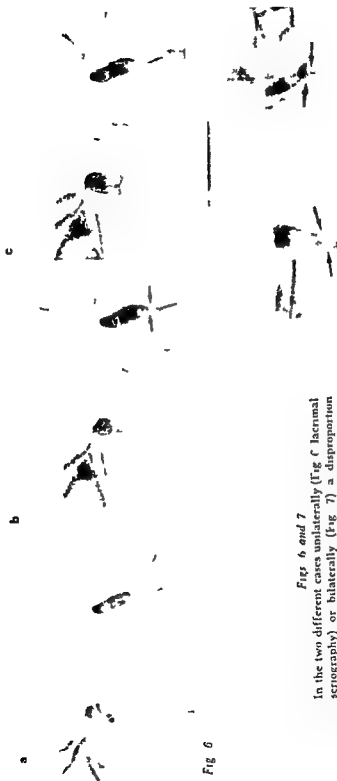


Fig 6

# *Figs 6 and 7*

In the two different cases unilaterally (Fig 6 lacrimal sacriography) or bilaterally (Fig 7) a disproportion ( $\rightarrow \leftarrow$ ) is noticeable between the diameter of the sac and that of the lumen below it (in Fig 6 on the right side a lachryostasis combined with a complete obstruction of the sac). In both cases the drainage is normal.



*Fig 8*



*Fig 9*

*Figs 8 and 9*

In two different cases an overall distended appearance of the right hand lacrimal passages is noticeable with delayed drainage into the nasal fossa (over 2 min)



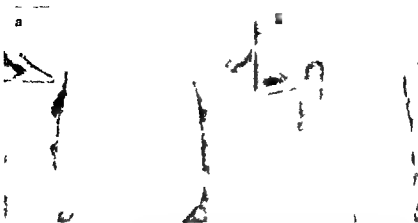


Fig 10 1 B

The lacrimal system show in both different cases dilated tracts alternating with strictured ones. Delayed drainage (3 min). In Fig 10B on the right side there is a complete obstruction of the sac.

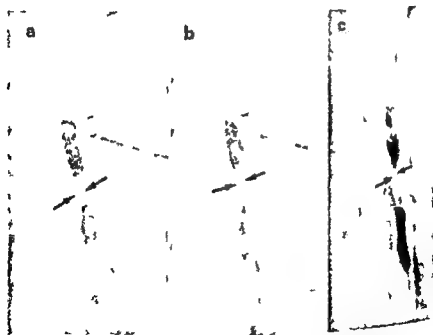


Fig 11

The sac appears moderately dilated and the drainage is retarded (3 min). A skip is noticeable in the filling of the lacrimal lumen and the point of passage between sac and duct never shows up in the various serigraphs (arrow).



*Fig 12*

drainage of the lacrimal passages is slowed (over three min) by stoppage of the contrast medium on the level of the lower tract of the lacrimo nasal duct

In the cases of functional disorders the sac does not appear to be particularly dilated on film. The radiological features are different in the various cases and may be summarized as follows

The sac appears to be of normal size but deformed and or barely deviated from the axis or sometimes also angled the asymmetry can be easier detected on comparison with the healthy side (Fig 5)

The sac shows a moderate dilatation which by itself may not be of biological significance and could go undetected by carefully examining the diameter of the lacrimal segments however it is found that a disproportion exists between the diameter of the sac and that of the downstream duct (Figs 8-9) without the picture changing in the various seriograms

The sac and the duct appear more or less dilated partly or completely sometimes presenting a frozen appearance (Figs 8-9) in other cases constricted sections alternate with wider ones (Fig 10)

The contrast medium skips a given tract (Fig 11) which therefore never becomes opacified

5) The sac and the lacrimo nasal duct are of regular shapes and exact drainage time is more or less prolonged (always longer than 3) seconds. Approximately in one third of our cases this fact constitutes the only pathological element and the contrast medium stops for such a time at a given level (Fig 12) or at several levels

## Discussion

Of the 100 lacrimal systems examined concerning patients affected by epiphora seventy presented probably purely functional disorders. In fact we can reasonably exclude anatomical lesions: such suspicion did not exist in clinical tests and the DCG showed neither dilatation nor remarkable deformations of the lacrimal pathways. On the other hand the existence of the co-existence of the morphological signs illustrated on Table I and the observation of the delayed drainage imply that the epiphora is due to disorders of functional nature. Naturally the morphological signs which have been described have a relative value as with all other signs in radiology. The detection of them can be useful because focuses attention on a functional pathology. The extended drainage time is of great importance for the diagnostic effects and yet this can be considered pathological only if it is longer than the exact normal time discharge of that particular contrast medium (Table I).

Now let us consider the causes responsible for the functional disorders. We may suppose there is some relation of functional disorders and anatomical alterations to inflammation at least a chronological one. The original cause may even be due to a slight inflammation of the lacrimal mucosa which does not provoke ectasias or deformations and is thus hardly remarkable on a clinical examination especially when this is performed according to the conventional technique. With time the inflammation stresses the functional disorders and these in turn enhance the inflammation. Afterwards through a mechanism similar to those occurring in other apparatuses (i.e. in the digestive tract) functional diseases may be transformed into organic ones. Inflammation of functional alterations further increase causing anatomical damage which can either be reversible or not.

At the DCG especially at the level of canaliculi the inflammatory foci are difficult to define. Furthermore as far as the drainage time is concerned we have few methods of evaluating this unless the serigraphic technique is adapted. On the contrary by means of the serial DCG with subtraction we can advance the diagnosis in the first period of early inflammation it is possible

to make the diagnosis in the second period of the functional disorders it is to make the diagnosis with the DCG then in the third period of anatomical alterations the diagnosis is *extremely easy* to make and can also be made by conventional dacryocystography

## Conclusions

Frequent reactions of the ophthalmologist – particularly if the canaliculi are involved – are characterized either by a discouraged lack of interest or by a scepticism about the chance of curing the epiphora. This attitude which noticeably affects the patient leads to inactivity or abstention from treatment. It finds its main support in the fact that the symptoms are neither severe nor painful though complications may become so. The initial difficulties connected with the epiphora are of an aesthetic nature and more or less well tolerated depending on the disposition of the patient. But since these cases offer good therapeutic possibilities this fatalism does not seem to be justified either by physician or by patient.

The treatment for the alterations sited in the canaliculi is largely surgical. The therapy of functional disorders in the sac and duct is often medical. After proper diagnostic recognition the causes responsible for the mucous hypertrophy and the epiphora can be approached more effectively. Occasional cases however these conditions are more the domain of a medical clinician than of a specialist (ENT Ophthalmologist) since abnormalities of lacrimal passage can sometimes be part of localisation of allergic or toxic disorders affecting the entire body.

A precise knowledge of the radiological features present in such disorders – and with increasing frequency as our experience expands – allows a better understanding of many cases of epiphora previously unrecognized since the previous diagnostic clinical and radiological examinations may be negative. It is desirable therefore that such functional disorders be recognized and better understood by both radiologists and clinicians alike. The former should arrive at the correct diagnosis using all investigative techniques and methods called for by the individual case while the clinicians should decide on the choice of treatment more likely to be therapeutically effective.

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# TEAR FLOW IN NORMAL HUMAN EYES DETERMINATION BY MEANS OF RADIOISOTOPE AND GAMMA CAMERA

BY

T SORENSEN and F TAAGEHOJ JENSEN

Tear flow was measured in normal human eyes by means of a radioisotope (technetium as pertechnetate in a normal saline solution) a gamma camera and a computer. By region of interest technique the elimination was shown to have two phases: an initial rapid elimination followed by a slower elimination after 5-7 min. The mean fractional turnover rate in the initial phase was  $0.197 \text{ min}^{-1}$  ( $n=35$  SEM = 0.013) and in the basal phase  $0.053 \text{ min}^{-1}$  ( $n=35$  SEM = 0.003). Assuming a constant tear volume these values corresponded to a tear flow of  $1.4 \mu\text{l min}^{-1}$  and  $0.6 \mu\text{l min}^{-1}$  respectively. There was no significant difference between the fractional turnover rate in the upright and supine position. No difference was found in tear flow between males and females. With the eyes closed the fractional turnover rate was low with intermittently rapid outflow of tears. Irritation to the contralateral eye with a filterpaper caused a stimulated tear flow of  $4.4 \mu\text{l min}^{-1}$ . A nomogram facilitating back ground corrections was constructed.

**Key words:** Tear flow - human - technetium - gamma camera - back ground correction

Measurements of tear secretion have been carried out by several authors with various results. Values from 12 to  $100 \mu\text{l/min}$  have been reckoned. The varying results are not surprising considering the conditions under which tears were collected. In methods using a filterpaper a stimulated tear secretion is measured. The amount of tears in the filterpaper is however highly dependent

n the outflow of tears through the tear drainage system and the amount is outflow is totally unknown. A stimulation to the eye will cause a rapid flow only for a few minutes (Mishima et al 1966 Sørensen & Taagehøj 1975). The varying results consequently depend on the time of measurement: the longer the determination time the smaller the influence of the initial high tear flow. In Table I are outlined the results of some previous studies. Schirmer (1903) is usually quoted having found a tear secretion of 15-20 gr/16 h. In fact he found 2-0.4 gr/16 h and an evaporation of 0.2 gr/16 h. When comparing Schirmer's results with the values in Table I the evaporation should not be included. According to later studies on rabbits (Mishima & Maurice 1961 and Iwata et al (1969) carried out under more physiological circumstances Schirmer's evaporation rate was almost three times too high. An excellent study on the dynamics of tear flow was presented by Mishima et al (1966) using fluorescein and a fluorophotometer. As a new finding they found that the elimination of fluorescein fairly well fitted to the exponential form of elimination which had been assumed in other studies (Kirschner & Brandt & Fritzsche 1967). Mishima et al also were first to demonstrate a biphasic elimination with an initial high flow and a lower flow after 4 to 5 minutes. In 1979 Rossomondo et al introduced lacrimal scintigraphy by means of a radioisotope and a gamma camera. Quantitative data on the dynamics of tears

*Table I*

Tear flow found by various authors. The values are not corrected for evaporation.

Author	Method	Tear flow ( $\mu\text{l}/\text{min}^{-1}$ )	Determination time
Schirmer (1903)	filter paper	0.2-0.4	0.15-2.5 hours
Lik (1957)	filter paper	15	5 min
Ver & Jaeger (1957)	dye dilution	10	2 min
Kirschner (1964)	dye dilution	0.3-0.9	0.5 hours
Mishima (1965)	dye dilution	10-20	5 min
Mishima et al (1966)	dye dilution	1.2	7-15 min after instillation
Brandt & Fritzsche (1967)	dye dilution	6.6	3 min
Wiersma (1967)	calculated	2.4	
Sørensen & Taagehøj (1975)	Tc dilution	0.6 (0.5 see text)	7.5-15 min. after instillation
(present study)			



**Table II**  
Tear flow studies using  $Tc^{99m}$  as tracer

Author	Vehicle	No of determinations/persons	Volume	Radioactivity	Evaluation
Dressler & Denffer (1974)	$Tc$ pertechnetate	not revealed	20 $\mu$ l	70 $\mu$ Ci	half time 1 min (in conjunctival sac)
Dressler & Denffer (1975)	$Tc$ sulphur colloid	10 eyes	10 $\mu$ l	50 $\mu$ Ci	half time 1 min (in conjunctival sac)
Carlton (1975)	$Tc$ (vehicle) not revealed	28 persons	15 $\mu$	100 $\mu$ Ci	half time 56 sec (in conjunctival sac) median transit time to the bottom of the nasolacrimal duct 43 sec (4-923)
Hardberger et al (1975)	$Tc$ pertechnetate in saline	4 eyes	3 $\mu$ l	not revealed	half time 4.6 min (region of interest not defined)
Hurwitz et al (1975)	$Tc$ sulphur colloid	7 persons	15 $\mu$ l	150 $\mu$ Ci	erect half time 4.1 min ( $n=7$ ) supine half time 38.0 min ( $n=7$ ) (region of interest was inner canthal area)
Myer & Dau ch (1975)	$Tc$ pertechnetate	73 persons	2 and 10 $\mu$ l	0 $\mu$ Ci	transit time in tear film 1.1 sec transit time in tear film 1.1 sec

a similar equipment have been published by several authors (Table II). The results are not directly comparable owing to differences in regions of interest, evaluation methods, vehicles and instilled volumes. Due to the elimination during first minutes after instillation the reckoned half times must be converted to a fractional turnover rate. However the tendency of the radioisotope introduced into the conjunctival sac very rapidly leaves via the tear pathways. This paper describes the results of dynamic tear flow studies in normal human eyes using a radioisotope and a gamma camera with computer assistance, the results being expressed as fractional turnover rates.

### Material

Volunteers were selected from the hospital staff, students and myopic persons who were later fitted with soft contact lenses. In 35 normal persons (males 20, females) a determination was carried out in one eye only. A second determination was performed in six persons with a few days interval, with only five min interval in 13 persons.

### Methods

In another paper (Sørensen & Taagehøj Jensen 1977) the method has been described in detail.

The patient was placed in a supine position with his eye approximately 3 cm from the hole collimator of the gamma camera, i.e. 30 cm from the scintillating crystal. 10  $\mu$ l of a normal saline solution containing approximately 900  $\mu$ Ci technetium ( $^{99m}$ Tc) as pertechnetate was placed on the center of the cornea without local anaesthesia. A scintigram was taken at the beginning and at the end of 15 min measuring time to unveil a possible displacement of the eye or trapping of the radioisotope in the lashes.

By serially radioactivity recordings at ten sec intervals from the designated conjunctival sac area (region of interest technique) an elimination curve was constructed by the computer and plotted on a semilogarithmic system. From the approximated curves in the initial and basal phases the fractional turnover rates were calculated (Sørensen & Taagehøj Jensen 1977) (See Fig 3).

In the investigations on subjects in the supine and upright position a newer equipment, not principally different from the detection system used in the other determinations, was utilized (new detection system: gamma camera Ohio nuclear ON-100, whole collimator interfaced minicomputer Varian 620/L-100). With this new detection system background corrections were not necessary.

In 9 persons a Schirmer filter paper reading was taken to correlate this estimation of tear secretion to the fractional turnover rate. The filterpaper standardised by Hal-

berg & Berens (1961) was placed in the lateral third of the lower lid and e.h. after five min or in patients with high lacrimation read as the total wetting of filterpaper (30 mm) per time required for the total wetting

The comparative tear flow determinations in the supine and upright position were separated by an interval of several days

The elimination of technetium was followed in nine normal persons placed in the supine position with closed eyes

In three persons the stimulated fractional turnover rates were determined by placing a filter paper strip in the fellow eye to the one being investigated

### Background correction

The contribution from the background components has been evaluated in another paper (Sørensen & Taagehøj Jensen 1971) In this study corrections were calculated for the natural background  $B_n$  and the background  $B_t$  due to the absorption through the conjunctiva and recirculation to the region of interest ( $B_t$  is the activity at time  $t$  and  $B_f$  the final value of  $B_t$  at time  $\infty$ )

The fractional turnover rate from the first determinations were corrected according to equation (1)

$$Q_t = Q_0 \cdot e^{-k_1 t} = A_0 \cdot e^{-k_1 t} + B_n + B_f (1 - e^{-0.015 t})$$

When double determinations with five minutes interval were done the second fractional turnover rate was corrected according to equation (2)

$$Q_t = Q_0 \cdot e^{-k_1 t} = A_0 \cdot e^{-k_1 t} + B_n + B_f (1 - e^{-0.015(t+\infty)}) + B_f (1 - e^{-0.015 t})$$

$Q_t$  is the recorded activity at time  $t$

$A_0$  the activity representing the deposit at time 0

$k$  the estimated fractional turnover rate on condition of no background components

$k_1$  the fractional turnover rate corrected for background components,

$t$  the time in minutes

$e$  the base of the natural logarithm and

0.015 = the fractional turnover rate found in absorption studies on normal persons (Sørensen & Taagehøj Jensen 1979)

The corrections for these background components is shown graphically in Fig 1

### Results

As shown in Fig 3 an initial very rapid elimination was found in the first 2 min following instillation due to the outflow of the surplus volume of tears. Then a steady state was present for a few min seen as a straight line on a semilogarithmic plot (= initial phase in Table III) The following part of the elimination from 7 to 15 min was called the basal phase. Because a steady state cannot be assumed in the first 1-2 min a tear flow has not been calculated for this phase

The fractional turnover rates in the initial and basal phases are shown in Table III and Figs 1 & 2 The mean basal fractional turnover rate was 0.015

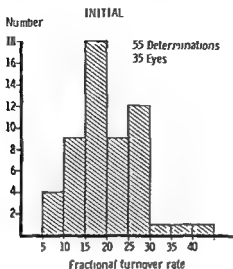


Fig 1

Fractional turnover rates in the initial phase in normal human eyes Dimension on abscissa ° • min<sup>-1</sup>

Table III

Normal values for fractional turnover rate

phase (closed eyes)

fractional turnover rate (one eye in 35 persons)	0.033 min <sup>-1</sup> (SEM 0.0034)
fractional turnover rate (one eye in 19 persons)	1 determination 0.035 min <sup>-1</sup> (SEM 0.0049)
	2 determination 0.04 min <sup>-1</sup> (SEM 0.0040)
	(t test for paired data $\alpha P = 0.10$ )

phase (open eyes)

fractional turnover rate (one eye in 35 persons)	0.197 min <sup>-1</sup> (SEM 0.013)
fractional turnover rate (one eye in 20 persons)	1 determination 0.203 min <sup>-1</sup> (SEM 0.019)
	2 determination 0.178 min <sup>-1</sup> (SEM 0.014)
	(t test for paired data $\alpha P = 1.00$ )

phase (open eyes)

fractional turnover rate (one eye in 9 persons)	0.043 min <sup>-1</sup> (SEM 0.011)
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elimination phase (closed eyes)

fractional turnover rate (one eye in 5 persons)	0.159 min <sup>-1</sup> (SEM 0.040)
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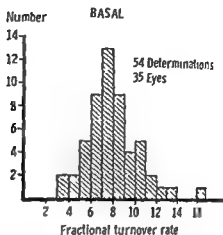


Fig 9

Fractional turnover rates in the basal phases in normal human eyes Dimensions abscissa % min<sup>-1</sup>

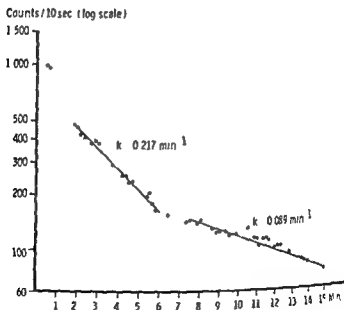


Fig 3

Activity time curve from the conjunctival sac area Dots represent radioactivity in ten second interval Solid lines are approximated curves The fractional turnover rates in the initial and basal phases are indicated with a  $k$  (In this example the background correction was high see Fig 10)

$0.053 \text{ min}^{-1}$  With the assumption of a tear volume of  $7 \mu\text{l}$  in the conjunctival sac (Mishima et al 1966) the tear flow could be calculated to be  $1.4 \mu\text{l min}^{-1}$  neglecting the absorption to the blood and the evaporation of water. The fractional turnover rate in the initial phase was found to be  $0.59 \text{ min}^{-1}$  corresponding to a tear flow of  $1.4 \mu\text{l min}^{-1}$ .

Biexponential elimination in a semilogarithmic plot was found in all 54 determinations with the exception of 3 that turned out to have a monophasic elimination. There was no obvious explanation for this monophasic elimination except in one patient who suffered watery eyes outdoors.

In double determinations the second determination had smaller fractional turnover rates especially in the initial phases ( $t$  test for paired data  $2p = 1-2\%$  to  $10\%$ ) for determinations with days and 5 min intervals. The difference was significant in double determinations with 5 min intervals ( $t$  test for paired data  $0.5-1.0\%$ ,  $n = 13$ ) and not significant with days interval between the measurements. In the basal phases this tendency was less pronounced ( $t$  test for paired data  $1-9\%$ ,  $p = 5-10\%$ ,  $n = 19$ ) for determinations with days and 5 min intervals and  $0.5-5.0\%$  ( $n = 13$ ) for 5 min interval determinations).

No difference was found between males and females neither in the initial phase nor in the basal phase. The range of age was 15-40 years (most of the persons were 20-30 years of age) and therefore the age flow correlation was not investigated.

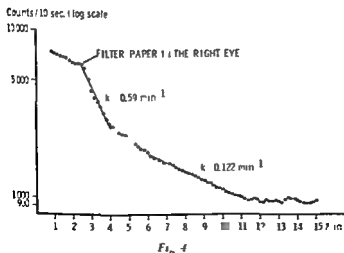
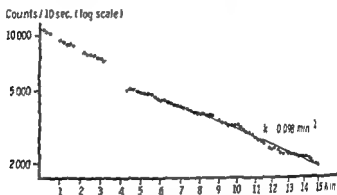


Fig. 4. Radioactivity time curve from a normal left eye during stimulation to the right eye by a filterpaper at the time marked by the arrow.

*Table IV*

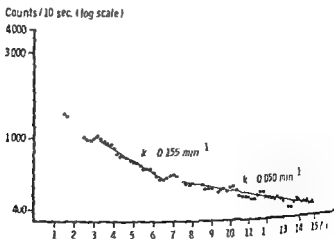
Fractional turnover rates in the supine and erect position in the same person  
Fractional turnover rate (mean  $\pm$  SEM)

	Supine	Erect
Basal (n = 7)	0.019 $\pm$ 0.008 (min <sup>-1</sup> )	0.063 $\pm$ 0.019 (min <sup>-1</sup> )
Initial (n = 7)	0.164 $\pm$ 0.015 (min <sup>-1</sup> )	0.147 $\pm$ 0.011 (min <sup>-1</sup> )



*Fig 5A*

Activity time curve from an eye not slightly turned to the side opposite to being investigated. Note the small fractional turnover rate and the less pronounced rapid outflow in the first seconds after instillation.



*Fig 5B*

From the same person as in Fig 5A the head being turned correctly

method caused only a small stimulation to the eye. A more stimulated flow was demonstrated by performing a Schirmer filterpaper test in the eye to the one being investigated. In Fig. 4 is the moment of the insertion of filterpaper marked by the arrow. The stimulated tear flow was  $4.4 \mu\text{l min}^{-1}$  (mean fractional turnover rate  $0.63 \text{ min}^{-1}$ ,  $n = 3$ ). The fractional turnover rates were determined in seven persons in the supine upright position in the same eye. The results are outlined in Table IV. There was no significant difference between the fractional turnover rates in the basal nor the initial phase (p. 0.1–0.2 and 0.4–0.5 respectively) described in an earlier paper (Sørensen & Taagehøj Jensen 1971) the eyes of the volunteers were slightly turned to the opposite side of the eye investigated to avoid pooling of the radioisotope in the outer canthal angle. If the head was placed with the nose perpendicular to the ceiling or slightly turned to the side of the investigated eye, an elimination curve with a biphasic shape was seen as illustrated in Figs 5A & 5B. The rapid outflow in the first 1–2 min is not present and the curve is not biphasic. This head tilt in the supine position was of paramount importance. With the

*Fig. 6A*

Scintigram of an eye without turning the head to one side





Fig 6B

Scintigram of the same eye with the head correctly slightly turned to the side opposite to the investigated eye

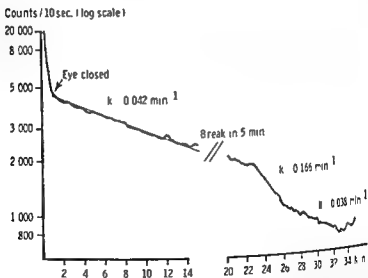
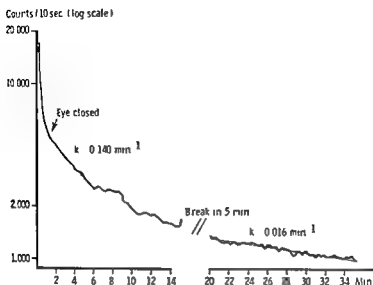


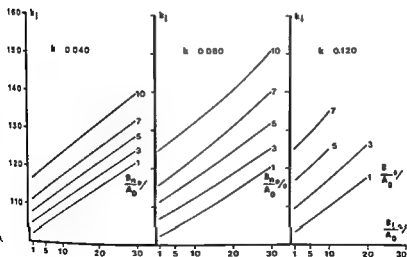
Fig 7

Activity time curve from a person with closed eyes. The person blinked frequently in the first minute. Then the eyes were closed for 34 min. A rapid elimination phase is seen from 23 to 26 min.



*Fig 8*

ed eye activity time curve from another person (see text in Fig 7) Rapid elution phase is less pronounced (9 to 10 min) Diphasic shape as in normal open eyes is seen with the change at 6 min after instillation.



*Fig 9*

ograms for background corrections Ordinate Corrected fractional turnover rate in percent of non corrected value Interpolation can be done for values not referred See text for further explanation

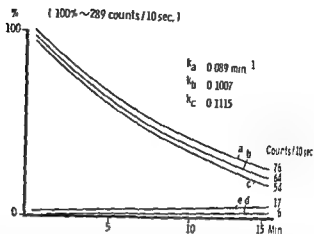


Fig 10

Graphical illustration of background correction (values from Fig 3). Curve a is the actual curve approximated to the actual curve in the basal phase. Curve c and d are the background curves according to equation (1) and (7). Curve b = a - d and curve e = a - c are the first and second determination respectively.

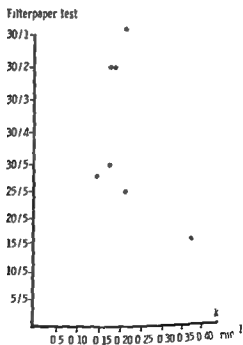


Fig 11

Correlation between filterpaper test and fractional turnover rate in the initial phase.

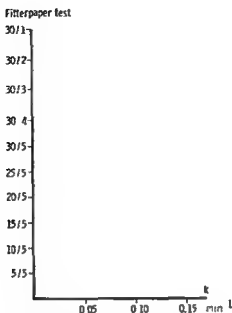


Fig 12

relation between filterpaper test and fractional turnover rate in the basal phase

id not turned to either side (Figs 6A & 6B) or turned to the wrong side the boetrium pooled in the outer canthal area resulting in irregular shaped curves. In the studies with closed eyes the persons were asked to blink frequently for out one min to blink out the surplus volume after installation. A diphasic type with a change of the fractional turnover rate at 3-6 min as in open eyes is seen in three persons (note change of elimination curve in Fig 8 after 3 min). In six persons no such change was seen after 3-6 min (Fig 7). The mean fractional turnover rate in these nine persons was found to be  $0.048 \pm 0.011 \text{ min}^{-1}$  mean  $\pm$  SEM (using the basal phase from 7 to 15 min in the three persons with a diphasic shape) corresponding to a tear flow of  $0.34 \mu\text{l min}^{-1}$  which is highly significant from tear flow in open eyes (t test  $2 P < 0.001$ ). In 5 of the 9 persons with closed eyes intermittent phases (rapid elimination phase in Table III) with a higher elimination rate were seen (in the person in Fig 7 from 23 to 26 min after instillation). The mean fractional turnover rate in these phases was found to be  $0.189 \pm 0.020 \text{ min}^{-1}$  (mean  $\pm$  SEM  $n = 5$ ). The difference between the rapid elimination phases and the basal phases in the closed eye situation was significant (t test  $2 P < 0.001$ ). In four persons rapid elimination phases were less pronounced or absent (in the person in Fig 8 a small rapid elimination phase is placed from 9 to 10 min after instillation).

Tear flow calculations beyond the 15 min measuring time was not carried out in persons with open eyes because the fractional turnover rates at this time approximated the values found after irrigation of the conjunctiva ( $1 \text{ min}^{-1}$  Sorensen & Taagchoj Jensen 1977). The phases with rapid elimination were found in the time interval 4–30 min after instillation. For technical reasons studies beyond 35 minutes have not been carried out.

The background corrections were calculated by the computer. To facilitate corrections in later studies and to illustrate the magnitude of the background corrections a nomogram was constructed using equations (1) and (2) (Fig. 1). There are three variables in these calculations. The natural background percent of  $A_0$  ( $\frac{B_a}{A_0} \%$ ) the background due to absorption and redistribution of the radioisotope ( $\frac{B_t}{A_0} \%$ ) and the fractional turnover rate  $\frac{B_0}{A_0} \%$  was found to be  $2.1\% \pm 0.3\%$  (mean  $\pm$  SEM) and  $\frac{B_t}{A_0} \%$  to be  $6.8\% \pm 1.0\%$  (mean  $\pm$  SEM). The fractional turnover rates in the initial phases were not corrected for background radiation since  $\frac{B_a}{A_0}$  and  $\frac{B_t}{A_0}$  were very small in this phase of the elimination.

In Fig. 10 is shown an example of the curves used in the correction. The basal phase in Fig. 3 has been used due to its high background correction. Curve b is the corrected curve when the example is from a single or first determination ( $b = a - d$ ) and curve c is the corrected curve when the example is from a second determination 5 min after the end of the first determination ( $c = a - e$ ).

The results of the filterpaper readings can be seen in Figs 11 & 12. Neither in the initial nor the basal phase was a correlation to the fractional turnover rate demonstrated.

## Discussion

The fractional turnover rate can be used as a parameter for tear flow. The tear flow  $L$  ( $\mu\text{l min}^{-1}$ ) can be calculated from  $L = k \cdot V$  where  $V$  is the tear volume in the conjunctival sac.

The tear volume has been determined by Mishima et al. (1966) to be  $10 \mu\text{l}$  whereas Zintz & Schilling (1964) found a value of  $23 \mu\text{l}$  in young and  $9 \mu\text{l}$  in old individuals. For reasons mentioned by Mishima et al. a tear volume of  $7 \mu\text{l}$  seems most reasonable. Furthermore they found that the tear volume

slightly dependent on tear flow (a ten fold increase in tear flow caused a doubling of the tear volume)

The amount of transport of the radioisotope through the conjunctiva to the general circulation also influence the calculation of tear flow. Sørensen & Schjø Jensen (1979) found a transport of  $1.5\% \text{ min}^{-1}$  of technetium per cent in normal human eyes. Whether this transport is accompanied by a water transport of the same magnitude is not known. If a water transport takes place the tear flow calculation needs no correction for the transconjunctival transport of technetium. If water is not transported the mean fractional turnover rate should be corrected from  $0.083 \text{ min}^{-1}$  to  $0.068 \text{ min}^{-1}$  and the tear flow from  $0.6 \mu\text{l min}^{-1}$  to  $0.5 \mu\text{l min}^{-1}$  (corrected for the half life of technetium  $t_{1/2} = 6 \text{ h}$ ).

The evaporation from the precorneal tear film has been found to  $0.1 \mu\text{l min}^{-1}$  in rabbits (Iwata et al 1969; Mishima & Maurice 1961) correcting the flow to 0.7 and  $0.6 \mu\text{l min}^{-1}$  respectively.

The difference between first and second determination was less pronounced in the basal phase. One explanation can be that the approximated background correction in the second determination was not high enough. The other explanation is that patient was more quiet during the second determination resulting in less reflex lacrimation. Consequently one would expect greatest difference in the initial phases which in fact is the case.

The interpretation of the biphasic shape of the curves has been that the instillation of technetium caused some stimulation to the eye resulting in an increased tear flow for approximately five min. This initial higher fractional turnover rate was not as high as that caused by the intense stimulation from a tearpaper in the fellow eye.

No difference was found between the fractional turnover rates in the supine and upright position. During the measurements the patients heads were turned approximately 20 degrees to the side opposite to the one being investigated. If no tilting of the head was omitted a pooling of the radioactivity at the outer orbital area was seen on the oscilloscope display resulting in irregular shaped curves and often an overflow out of the lateral canthus. The advantage of performing the investigation in the supine position was the steady fixation of the head which was not easily obtained in the upright position for 15 min.

Differences between the erect and supine position was found by Hurwitz et al (1975) on seven normal persons. The authors did however apparently not tilt the patients head and the difference is therefore understandable. Furthermore their region of interest was the inner canthus - not the whole conjunctival sac. Generally the gravitational force is considered of some importance in tear drainage. This assumption seems thus only valid when the

outer canthus is placed at the same level or lower than the inner canthus in accordance with the observation made by Maurice (1953). Even when a drop of 10  $\mu$ l injected into the eye will fall to the outer canthus and there despite strong continuous blinking. Figs 6A & 6B show the pattern of radioactivity at the outer canthal area.

The explanation of the intermittent high fractional turnover rate in the eyes is not obvious. An intermittently high tear secretion would not be a high fractional turnover rate without a simultaneous rapid drainage of secreted tears. Therefore intermittently high outflow must take place in the closed eye situation in some persons with or without a simultaneously high secretion. The rapid outflow can not be explained by a few blinks in the rapid outflow continued for 1-5 min.

The filterpaper readings showed that the Schirmer test apparently has a low value in normal persons.

Mishima et al (1966) found a tear flow twice as big as in this study. Their region of interest was a small area in the lower marginal tear strip whereas our region of interest was the entire conjunctival sac. By Mishima's diffusion method a correction for fluorescein diffusion to the general circulation was not calculated whereas the present method yielded a tear flow compensated for the conjunctival transport of technetium.

## Acknowledgment

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# PHAGOCYTOSIS OF LATEX MICROSPHERES BY THE EPITHELIAL CELLS OF THE GUINEA PIG CONJUNCTIVA

BY

STEFAN LATKOVIC and SVEN ERIK G NILSSON

The capability of conjunctival epithelial cells to phagocytize part of foreign matter was studied experimentally in the guinea pig. Permeable conjunctival epithelium was prepared for transmission electron microscopy by perfusion fixation 4 and 24 h after instillation of latex microspheres (0.79  $\mu$  in diameter) into the lower conjunctival fornix. A varied number of microspheres were internalized by the superficial epithelial cells in both cases. The intracellular microspheres single (4 and 24 h) or aggregated (only at 24 h) were always membrane enclosed corresponding to primary phagosomes. Some indications of secondary phagosomes were seen as well. In the 24 h specimens microspheres were present also in the intermediate epithelial cells implying a transfer from superficial to deeper cells. Thus superficial as well as intermediate epithelial cells of the guinea pig conjunctiva are capable of active phagocytosis of inert particles.

**Key words:** conjunctival epithelium - phagocytosis - latex microspheres - transmission electron microscopy

The polymorphonuclear leukocytes and the mononuclear phagocytes are generally regarded as the professional phagocytizing cells (Stossel 1974). A number of recent studies demonstrated that other types of cells not characterized as phagocytes are capable of internalizing bacteria or particles after by a process resembling phagocytosis: epidermal keratinocytes (Hof & Konrad 1972), retinal pigment epithelium (Hollyfield & Ward 1974, Fér & Mixon 1976), epitheloid HeLa cells (Bovallius & Nilsson 1975, Ahlström

wic 1978) muscle cells (Garfield et al 1975) and mucosa of the genital (Ward et al 1975). Intracellularly located bacteria were also observed in conjunctival epithelial cells (Racz & Tenner 1963, Tenner et al 1971, Zimianski et al 1974) and the process was regarded as endocytosis/phagocytosis. In the case of bacteria found within the epithelial cells opinions differ as to the process by which the bacteria pass across the cell membrane. While some workers regard it as phagocytosis (Zimianski et al 1974, Bovallius & Nilsson 1975, Ward et al 1975, Kihlstrom & Latkovic 1978) others consider it as transmembrane penetration through the cell membrane (Takeuchi & Sprinz 1968, Takeuchi et al 1968, Takeuchi 1975, Ward et al 1974, Gemski & Formal 1975). In the present study was undertaken to determine whether conjunctival epithelial (CE) cells are capable of phagocytosis. Using latex microspheres as a metabolic, non toxic tracer instead of biological material it was possible to minimize the influence of the material to be phagocytized and thus in a simplified system study the responses of the phagocytizing cells. For the ultrastructure of the normal perilimbal conjunctival epithelium of guinea pig the reader is referred to Latkovic & Nilsson (1979a, b).

## Material and Methods

On clinically healthy adult pigmented guinea pigs were anaesthetized intraperitoneally with 20 mg/kg b.w. sodium pentobarbital (Nembutal® Abbott) and the eyelids closed with agraffes. Latex microspheres 0.794  $\mu$  in diameter (Dow Chemical) were introduced in a 10% suspension (weight/volume) into the lower conjunctival fornix of each eye by instilling approximately 0.05 ml through a needle with a rounded point. After 4 h one of the animals was perfused via the left ventricle with 4% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2. Each eye was removed in one piece together with the eyelids and fixation was continued for 72 h by immersion in glutaraldehyde solution as above but at 4°C. The second animal was kept lightly anaesthetized by repeated small doses of sodium pentobarbital. Every 4 h 0.05 ml of latex suspension was instilled into the lower fornix of each eye. After 24 h the second animal was perfused and the eyes excised and fixation performed as above. Parallel sided strips of conjunctiva from the limbus to the margin of the lower eyelid were dehydrated in increasing concentrations of ethanol and embedded in Epon. Thin sections of conjunctival epithelium from the perilimbal zone were cut at right angles to the limbus. For further details see Latkovic & Nilsson (1979a).

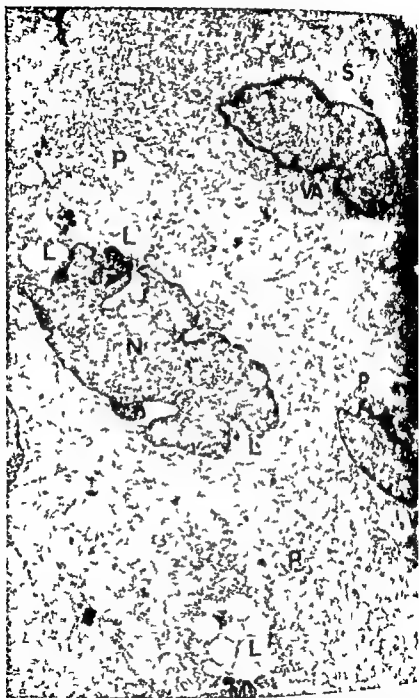


Fig. 1

Survey electron micrograph of guinea pig conjunctival epithelium from the per zone slightly obliquely cut demonstrating latex microspheres (L) as single or in aggregates in polyhedral (P) cells of the intermediate layers of melanocytes (N nucleus S superficial cell VA vacuole (24 h incubation with latex particles).



Fig 2

Primary phagosome with a single latex microsphere (L) in the apical part of a superficial cell. An autophagic vacuole is seen to the left of the microsphere. VE = vesicles (4 h incubation)  $\times 47,600$

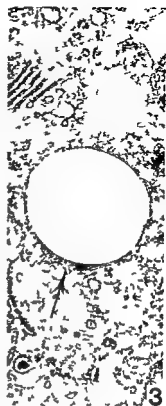


Fig 3

Profile of what may be a secondary phagosome containing a single latex microsphere and some electron dense material (arrow) within the membrane (4 h incubation)  $\times 43,300$

## Observations

Profiles of latex microspheres very low in electron density rather uniform in size and round or slightly oval in shape were easily identified (Fig 1). The latex substance of the microsphere was extracted to a large extent but a residue was often seen as an irregular area of somewhat higher electron density (Figs 1, 2 and 4).

The CF cells showed normal ultrastructural characteristics without any signs of damage.



**Fig 4**

Two secondary phagosomes in the perinuclear zone of an epithelial cell. Two latex microspheres (L) respectively are visible in the section. Arrows point to the surrounding membrane. Note the granulated electron dense material between and around the microspheres. N nucleus MI mitochondria VA vacuole (24 h incubation) x25,000.

No latex microspheres were observed attached to the free surface of the epithelium. The number of microsphere containing cells per section and the number of microspheres per cell varied within a wide range in both experiments.

After 4 h incubation with latex suspension the microspheres were found only in the superficial CE cells. They were observed as single units at various levels within the cell. The most superficial microspheres were located approximately one microsphere diameter beneath the free surface (Fig. 5) while the deeper ones were most often seen in the perinuclear area. All microspheres

bound by a membrane usually tightly fitting but sometimes with narrow spaces which in certain cases could be filled with an electron dense material (Fig 3)

In the eyes subjected to fixation 24 h after introduction of latex suspension in the inferior fornix the distribution of microspheres within the cells was rather different. The number of microspheres in the superficial cells was fewer compared to the 4 h experiment and they were observed even in the polyhedral cells of the intermediate layers. Both in the superficial and the polyhedral CE cells the microspheres were found either as single units or as small aggregates (Figs 1 and 4). In addition to microspheres in different numbers the membrane sometimes also enclosed finely granulated material (Fig 4) or small vesicles. Melanosomes could also be included in the aggregates (Fig 1). Although microspheres were seen in the apical cell compartment most of them were concentrated in the perinuclear zone especially in the polyhedral intermediate cells and not infrequently in the vicinity of nuclear indentations (Figs 1 and 4).

## Discussion

The phagocytic capability of CE cells was suggested in studies with different strains of bacteria (Racz & Tenner 1963; Tenner et al 1961; Zimianski et al 1964). The results of these investigations were not quite conclusive since the mode of bacterial entry into the cells could be either active penetration or pinocytosis.

The present study was undertaken to determine whether unstimulated CE cells are capable of phagocytosis. The possible influence on the phagocytizing cells of the material presented for phagocytosis was minimized by using microspheres of latex, a non metabolic substance which was found to be non toxic to the host cells in a number of *in vitro* and *in vivo* studies (Schoenberg et al 1961; Korn & Weisman 1967; Wolff & Konrad 1972; Garfield et al 1975; Hollyfield 1976; Feeney & Mixon 1976; Hollyfield & Ward (1974) however reported a toxic effect of latex microspheres on the retinal pigment epithelium of the *Rana pipiens* embryo but not of the tadpole or of the frog. Latex microspheres are easily identified in the electron microscope.

Attachment of particles to the cell membrane initiates the subsequent stages of internalization (Simson & Spicer 1973). Since latex microspheres were observed within CE cells it can be deduced that they had been attached to the cell membrane prior to uptake. The fact that no microspheres were found attached to the free cell surface in our material may be explained by dissolution

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# CONTRACTILE PROPERTIES OF EXTRACULAR CLE IN CATS REARED WITH MONOCULAR LID CLOSURE AND ARTIFICIAL SQUINT

BY

GUNNAR LENNERSTRAND AND JERKER HANSON

Animals were raised with amblyopia by monocular lid suture or esotropia  
y transection of extraocular muscles in one eye at the age of 2-3 weeks  
ometric contractions in the inferior oblique muscle were recorded at 20  
weeks or at adult age and the results were compared with those of sim-  
larly aged cats with normal visual development. The speed of contraction  
nd the fatigue resistance was reduced in the lid sutured animals. In the  
otropic cats studied, fatigue resistance was also reduced but the speed  
f contraction did not change much.

It is suggested that the postnatal eye muscle development can be modified  
y manipulation of the visual input at an early age. Presumably the im-  
paired binocular vision in the lid sutured and esotropic cats reduced the  
demand for fusion vergences and this might be reflected in the changes  
of eye muscle properties. In monocularly lid sutured cats, eye muscle  
changes were the same on both sides, suggesting that amblyopia *per se* did  
not affect eye muscle development.

**Key words:** Extraocular muscle - cats - contractile properties - mono-  
lateral lid suture - artificial squint - binocular deficiency

Functions of most mammals are subnormal at birth and are subject to a  
postnatal improvement. In the cat, visual acuity and binocular vision  
have reached maximal capacity at the age of 10 to 20 weeks, the period  
of most rapid change occurring between 3 and 6 weeks of age (Blakemore  
Sluyters 1975; Mitchell et al. 1976). Oculomotor functions employed for

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fixation and binocular vision are probably fully developed in the cat at the age as indicated by a study of the postnatal eye muscle development in the kitten (Lennerstrand & Hanson 1978 *a* & *b*).

Numerous investigators have shown that the binocular functions of the eye can be severely impaired by monocular deprivation or artificial squint made before or during the most sensitive period of visual development, i.e. the age of 3-6 weeks (see Blakemore 1977). It has not been demonstrated, however, whether the binocular defects are accompanied by any changes in the contractile properties. We have compared contractile properties of inferior oblique muscles from normal cats and cats with monocular lid suture and artificial squint. The speed of muscle contraction and the fatigue properties seemed to be influenced by the operations which were performed before the sensitive period was over previously.

The results have been reported elsewhere in a preliminary form (Lennerstrand & Hanson 1978 *c*).

## Methods

The experiments were performed on four normal cats and ten cats from three litters with impaired binocular vision. These cats were raised in the same environment. Additional data have been obtained from two adult, normal cats, also from the same study of normal eye muscle development (Lennerstrand & Hanson 1978 *a* & *b*). Operations on the cats with binocular deficiencies were performed at the age of 3-6 weeks. Under ketamine anaesthesia either (1) the left eye was to be closed by suturing first the nictitating membrane to the upper fornix and then the lids to each other or (2) a left sided convergent squint was produced by transsection of the lateral rectus muscle and the lateral slips of the retractor bulbi muscle of the left eye. A necessary repair of the lid closure was done within one day. One group of cats, consisting of two normal, one esotropic and two lid sutured cats, were examined at the age of 70 weeks. Another group of four normal, two strabismic and two lid sutured cats were examined when they were adult (11-15 months of age). Of the ten muscles sectioned, only those with clear esotropia (convergent squint) were examined physiologically. The experiments were performed on the inferior oblique muscle of the unoperated right eye in strabismic cats; the surgical trauma might have affected the inferior oblique function on the operated side. In lid sutured and normal cats, the inferior oblique function on both sides were used.

The experimental technique has been described in detail in previous papers (Lennerstrand & Lennerstrand 1977; Lennerstrand & Hanson 1978 *a* & *b*). Isometric tension measurements were made with the muscle attached to a sensitive force measuring system, the measuring element being a semiconduction transducer (Pixie 8101, Endevco Laboratories). The muscle was activated by nerve stimulation with bipolar platinum electrodes. Stimulus pulses of 0.1 msec duration were delivered singly or in trains from a Grass S8 stimulator. Twitch characteristics, tetanic tension development, fatigue properties and

behaviour were studied in the same manner as previously described. All measurements were done with the muscle at optimal length for twitch contraction. Physiological results from the two eyes of the same animal were compared with statistics in normal cats and lid sutured animals. Data from normal and lid sutured cats were compared with *t* statistics for two means. The amount of data on 8 cats in each age group was too small to be statistically assessed.

## Results

The contractile characteristics of inferior oblique muscles of the two eyes were compared in lid sutured cats and in normal animals. No statistically significant differences were found within each group for any of the parameters studied. This held true both for the kittens 20 weeks of age and the adult cats. Findings justified that data from both eyes were lumped in the comparison between normal and lid sutured cats. The differences in contractile properties between muscles of normal, lid sutured and esotropic cats have been summarized in Table I. For all other contractile properties studied the values were the same in normal and operated animals. It was found that in adult cats contraction time (*ct*) and half relaxation time (*hrt*) were longer in lid sutured than in normal animals, i.e. the muscles of adult lid sutured cats contracted slower than the normal adult eye muscles (Fig. 1 and Table I). There was to be very little difference in this respect between adult esotropic cats and normal cats, or between 20 weeks old lid operated and normal animals (Table I). The maximal rate of tension rise during tetanic contraction was higher in lid sutured than in normal animals of the adult age, but this difference was not statistically significant. Maximal tension in twitch contraction was the same in all groups of the same age.

No difference between the normal and the lid operated animals were observed in the fatigue properties of the muscles. As shown in Fig. 2 the drop in tension during continuous activation for 30 sec was faster and deeper for lid sutured than for normal animals, i.e. the muscles of lid sutured animals showed a higher susceptibility to fatigue than muscles of normal animals both at kitten and at adult age. The differences were statistically significant for stimulation frequencies of 50 and 100 Hz (Table I) but not for 200 Hz. The same was found for the two adult esotropic cats studied.

The recovery of the twitch response from a 30 sec fatiguing stimulation was slower in the lid operated adult animals than in adult normal cats. This was most prominent in the 100 Hz and 200 Hz stimulations. The depression of twitch amplitude was more profound and lasted longer in operated than in

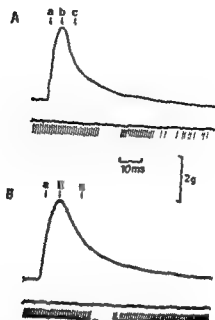


Fig 1

Isometric twitch responses (top traces) to supramaximal nerve stimulation in a cat with normal binocularity (A) and with monocular lid suture (B). Arrows indicate: (a) peak (b) and point of half relaxation (c) of the twitch contraction. Time interval a-b is contraction time (ct) and time interval b-c is half relaxation time (hrt). Lower traces for stimulus and time marking.

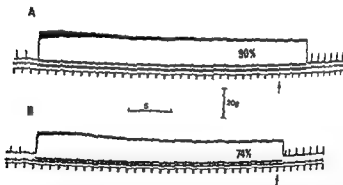


Fig 2

Endurance of adult inferior oblique muscles in a normal (A) and a monocular lid sutured cat (B). Top traces show muscle tension to the extreme left and right responses interrupted by a period of continuous 100 Hz stimulation. Fatigue resistance has been measured as the amount of tension in per cent of the initial tetanic tension remaining after 30 sec (arrow). The fatigue resistance is higher in the normal cat than in the lid sutured cat (74%).

Middle and bottom traces used for stimulus and time marking respectively.

For the first three (1, 2, and 3) and for the last two (4 and 5) were determined for twitches obtained 5 min after a 90 sec continuous tetanization at the rates of 1 and 2 sec respectively. The values have been given in percent of the corresponding pre-tetanic value.

	Pre-tetanic			Tetanic		
	N (n=4)	I (n=4)	P	N (n=8)	L (n=10)	E (n=2)
Twitch						
at (ms)	70 ± 0.3	70 ± 0.9	-	57 ± 0.6	69 ± 1.0	59
hrt (ms)	114 ± 2.3	92 ± 1.7	-	70 ± 1.0	96 ± 0.6	80
Endurance (%)						
at 50 Hz	139 ± 0.6	96 ± 0.0	*	100 ± 3.5	112 ± 0.0	106
at 100 Hz	79 ± 7	57 ± 1.5	*	90 ± 1.5	90 ± 0.9	■
Posttetanic						
twitch 100 Hz						
ampl (%)	91 ± 7	91 ± 4	-	90 ± 1.5	70 ± 6	72
hrt (%)	145 ± 0.7	100 ± 1.0	-	102 ± 1.0	119 ± 1.6	103
Posttetanic						
twitch 500 Hz						
ampl (%)	94 ± 8	90 ± 2.6	-	99 ± 9	62 ± 8	79
hrt (%)	190 ± 4.4	154 ± 3.4	-	105 ± 0.0	149 ± 4.6	195

- P > 0.05      \* 0.05 > P > 0.07      \* 0.02 > P > 0.01      \*\*\* P < 0.01

normal animals and the changes in *hrt* were more marked in operated cats shown in Table I for values obtained 5 min after the fatiguing stimulus. On the other hand the same time course of recovery was seen in both lid sutured and normal cats for the tetanic response to 200 Hz for 0.5 sec. The same tendency applied to results from esotropic cats. In animals 70 weeks of age the speed and tetanic recovery was the same for normal and operated groups.

## Discussion

This study has shown that manipulation of the visual input for kittens affects not only the visual but also the oculomotor development. Monolateral lid suture and acquired squint at an early age have been shown to change the properties of neurons in the visual cortex (see e.g. Blakemore 1977).

However, it has not been previously known that these procedures can induce changes in the speed of twitch contraction or the fatigue resistance of eye muscles. The cats with monolateral visual deprivation due to lid suture showed eye muscle changes of the same degree in the seeing and the closed eye. Thus it seems unlikely that the variations in eye muscle properties between normal and lid sutured cats were caused by monocular amblyopia. Instead it may appear that the changes seen in operated cats were in some way correlated with the state of their binocular vision, since the eye muscle changes seemed more marked in lid sutured cats than in the squinting cats.

Both the speed of contraction and the fatigue resistance were affected in the lid sutured animals but only fatigue properties in esotropic cats. Binocular vision is completely impeded in animals with monocular lid suture while in esotropic cats probably some peripheral binocularity remained. The conclusions were considered normal with regard to binocular vision. The conclusions concerning binocularity were inferred from data obtained in studies by other investigators on the visual cortex of normal cats (Hubel & Wiesel 1962, 1963) of cats with monocular visual deprivation (Hubel & Wiesel 1963, Blakemore & van Sluyters 1974) and of cats with acquired esotropia (Hanson 1976).

If binocular vision is defective or lacking the demand for fusion vergence eye movements is probably very much reduced. It may be speculated that the decreased demand affected the development of eye muscle fibers leading to the changes seen in eye muscle properties. The slow fiber system (Hess & Fuchs 1963, Matyushkin 1964, Bach & Raita & Ito 1966) presumably responsible for slow motor acts like fusion vergences (Lennerstrand 1975) would seem preferentially engaged. The lower fatigue resistance of eye muscles in operated animals of all ages tested would support this idea. Earlier studies indicated that

a fiber system developed earlier than the fast fiber system (Lenner & Hanson 1978 a b) and that it had reached a high degree of maturity cats during the so called sensitive period of the visual development very precise control of eye mobility is needed. However the speed of contraction was lowered in adult cats with lid suture but not in 20 weeks old suggesting that also the fast fiber system evolved differently in the later stages of development.

It might be of interest to compare the changes in eye muscle properties with changes in fast and slow skeletal muscle by disuse although the methods of immobilization of a limb or denervation of the muscles used in the skeletal muscle studies would seem to limit muscle function much more drastically than the selective abolition of fusion vergences in this study. It should also be kept in mind that eye muscles have a specialized fiber composition with a high proportion of slow fibers in skeletal muscle (Alvarado & van Horn 1975) which makes a direct comparison with other muscles difficult.

As a result of the present experimental technique in adult cat slow and fast hind limb muscles show reduced speed of contraction (Syrový et al 1972 Kean et al 1974). Prolonged inactivity induced similar changes in cat muscles (Davis & Montgomery 1977). Immobilization does not lead to any significant changes in the speed of contraction in hind limb muscles of the guinea pig (Maier et al 1976). The present experimental technique with a proposed loss of fusion vergences may be considered equivalent to partial denervation but not to an immobilization. Disuse of certain eye muscles may explain the reduction in speed of contraction that occurs after monocular lid suture. It is also well known that disuse lowers the contractile potential and the fatigue resistance of a muscle (Hooloszy & Booth 1967) and this might have occurred also in the eye muscles of lid sutured and denervated animals. In newborn animals disuse by denervation prevents the normal muscle fiber differentiation (Karpati & Engel 1967 Shafiq et al 1972). It is planned to expand the investigation by examining the same eye muscles with histochemical techniques and compare fiber composition and enzymatic profiles of these muscles in normal and binocularly impaired cats.

The present experimental technique has offered unique possibilities to study the effects of disuse on muscle development of a limited but well defined change in functional demands on a motor system. Thus it has been shown that disuse of oculomotor functions in this case probably restricted to fusional vergences may induce significant changes in eye muscle properties. From a clinical standpoint the results may prove of importance for clinical research on strabismic amblyopia. It is not clear whether the lack of the muscular substrate for fusion vergences might make proper eye alignment impossible in spite of a sensory cure through optical treatment.

## Acknowledgment

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# A CLINICAL STUDY ON THE DETECTION OF STRABISMUS ANISOMETROPIA OR AMETROPIA OF CHILDREN BY SIMULTANEOUS PHOTOGRAPHY OF THE CORNEAL AND THE FUNDUS REFLEXES

BY

KARI KAAKINEN and VEIKKO TOMMILA

Twenty two strabismus and 106 straight eyed patients with anatomically normal eyes were first photographed with a conventional camera equipped with a weak 100 mm teleobjective and coaxial flashlight and then examined clinically. The possibility of detecting strabismus anisometropia and ametropias in the photographs by noting the localisation of the corneal reflexes and examining the appearance and lightness of the fundus reflexes and their possible asymmetry were tested in a double blind study. Even small angled strabismus cases could be found because of the asymmetrical localisation of the corneal reflexes. In 18 of the 22 strabismus cases (82%) there was asymmetrical lightness of the fundus reflexes and the fundus reflex of the deviating eye was lighter than that of the fixating eye. All the straight eyed anisometropias of 3.0 diopters or more (free cases) were observed in the photographs because of the asymmetrical appearance of the fundus reflexes. In straight eyed anisometropias of under 3.0 diopters the fundus reflexes were symmetrical in 90 cases and asymmetrical in 11 cases (11%).

Only three out of eight hyperopias of from +4.5 to +6.0 diopters were found because of the light crescent in the low part of the pupil. All myopias of over -4.0 diopters (14 cases) were observed because of the light crescent appearance in the upper part of the pupil. No pupillary crescents appeared with refractions of less than -1.5 diopters myopia or less than +4.5 diopters hyperopia. 179 eyes came within this range. Even a technician can perform without premedication the method tested here for rapid and simple screening to detect strabismus and straight eyed

anisometropias of 3.0 diopters or more in small children or other patients who do not co operate well in normal clinical examination. Over -4.0 diopters myopias can also be found. The method was rather unreliable for finding hyperopias presumably because no cycloplegic drops were used.

**Key words:** strabismus - hyperopia - myopia - anisometropia - fundus reflex - corneal reflex - photographic screening of vision defects and eye disorders in children

In an earlier article (Kaakinen 1979) the author presented a simple method for strabismus anisometropia or ametropia screening of children by photography with a conventional camera and flashlight the corneal and the fundus reflexes simultaneously. The method and some experience of illustrative cases presented in that first part of the investigation. To establish the reliability of the method the results were compared with the test synoptophoric examinations and refraction measurements in the total strabismus and refractive error material in a double blind study. The aim of the investigation was to compare the sensitivity of the photography with the clinical cover test in quite small angled strabismus cases too. It can be rather difficult to find especially in small children. Straight eyed children with great anisometropic or ametropic refractive errors which may cause strabismus or need refractive correction were also considered as possibilities of imitations.

## Material and Methods

Total material was 128 patients. 22 of them were strabismus patients of the Helsinki University Eye Clinic. The remaining 106 patients were cases with eye examinations sent by the school health sister for ophthalmological examination. The average age of the patients was eight years because the latter group of patients consisted of pupils from the first and second grade of elementary school.

The patients were first photographed from a distance of one metre as described before (Kaakinen 1979). No mydriatic eyedrops were used before photography.

After photography a careful cover test in near fixation was performed. The direction of movement of the deviating eye in the cover test was expressed as easily observable or very small. The latter indicated a just discernible move-

ment mostly less than  $5^\circ$ . Heterophorias were not mentioned because they were naturally not to be found in the photographs. After the cover test was finished 1% cyclopentolatehydrochloride (Cyclogyl<sup>®</sup>) was instilled in the eyes and the examination was repeated after five min. The objective refraction was then established half an hour with a streak retinoscope. The transparent ocular media were searched with a slitlamp biomicroscope and an ophthalmoscope by the brightness of the fundus reflex. The fundus examination was performed at the same time with an ophthalmoscope. The synoptophoric angles were measured at the same time in the strabismus patients.

An experienced strabismologist and a student nurse examined the photographs separately. Neither knew the results of the clinical examination of the patients. They were first shown model photographs from the previous examination taken by the same method (straight eyes, very small eso- and exotropia,  $5^\circ$  and some larger eye deviations). They were asked to examine the photographs showing symmetrical and asymmetrical localisation of the corneal reflexes. Special attention was to be paid to very small asymmetrical localisation of the corneal reflexes. If uncertain it could be denoted with  $\pm$  (presumably asymmetrical = strabismus suspect). The colour or the lightness of the both corneal reflexes or the size and localisation of the possible light crescents in the fundus were to be noted as equal or asymmetrical. If the fundus reflexes were asymmetrical the lighter fundus reflex should be marked. Noticeable anisocoria should be marked.

The results from the examinations of the photographs were compared with the clinical examinations of the patients. In the case of the astigmatic refractive errors spherical equivalents were used.

## *Results*

All the patients had normal eyes in the fundus examinations. The transparent ocular media were clear in every case on both microscopical and ophthalmoscopic examination.

### *Strabismus (Table 1)*

Twenty two of the 128 patients had a strabismus with the cover test. The angle of the strabismus was  $\leq 5^\circ$  with a synoptophore in 12 cases, in all of the cases the angle with the cover test was indicated as very small. In addition there were two cases with a very small angle in the cover test in which the synoptophoric angles were  $+7^\circ$  and  $+13^\circ$ . In one case showing very small right hyper-

ie cover test the synoptophoric measurement was impossible because the as only one year old

etrical corneal reflexes in the photographs led the experienced ologist to identify 20 of the 22 strabismus cases. The student nurse the same result except that she observed four false strabismus sus

one false positive strabismus in the photographs that were straight he strabismologist had no false strabismus suspects and thus all his 106 ical corneal reflex cases had straight eyes with the cover test. Both ismologist and the student nurse found one +2 esotropia and one -2

as strabismus suspects and student nurse also had one strabismus t case with +4 esotropia. These were the strabismus cases that were ted from the photographs

er could find the two 1 esotropia cases in the photographs by examin localisation of the corneal reflexes but one was detected anyway se of the asymmetrical fundus reflexes and another because of the oria that was visible in the photograph. These changes were noted by the smologist and the student nurse. The strabismologist spent an average of nds in examining each photograph and student nurse about two s longer

#### smus and anisometropia (Table I)

trabismologist and student nurse obtained identical results in examining mmetry or asymmetry of the fundus reflexes. Eighteen of the strabismus had asymmetrical fundus reflexes and in all of them the fundus reflex e deviating eye was lighter than that of the fixating eye. Eleven of the smus cases had 0-1.4 diopters anisometropia and eight of these had metrical fundus reflexes. The anisometropia was over 1.4 diopters in 11 10 of them had asymmetrical fundus reflexes. Hence four strabismus cases ymmetrical fundus reflexes and the angle of strabismus in all of them ery small ( $\leq 4^\circ$ ). The pupils also were quite small in these cases

#### cases and anisometropia (Table II)

of 106 straight eyed cases 94 had anisometropia of between 0 and 1.4 ters 85 (90 %) of them had symmetrical and 9 (10 %) asymmetrical fundus

Two of the nine asymmetrical cases had anisocoria and a darker reflex on the smaller pupil side. In three cases the myopical crescents not quite equal in size which was the reason for the asymmetrical ap of the fundus reflexes. The anisometropia in these three cases was 0.3 and one without anisometropia. In two cases only were the fundus asymmetrical without anisometropia at all

Table I  
Strabismus cases

case No	cover test	synoptophore	anisometropia	Photographic results			
				strabismologist		nurse student	
				corneal reflexes	fundus reflexes	corneal reflexes	fundus reflexes
1	very small left exotropia	- 3°	45	+	+	+	+
2	marked left exotropia	+ 14°	10	+	+	+	+
3	marked left exotropia	+ 6°	43	+	+	+	+
4	very small left exotropia	+ 2°	03	+	+	+	+
5	very small right hyliotropia	± 0° vertical	00	+	+	+	+
6	marked right exotropia	+ 11°	01	+	-	+	+
7	very small right exotropia	3	00	+	+	+	-
8	marked right exotropia	+ 7°	18	+	+	+	+
9	marked right exotropia	+ 17	5	+	+	+	+

no	clinical picture	+ 33	5	+	+	+
13	very small right esotropia	15°	00	+	+	+
14	very small left esotropia	+ 4°	19	+	+	+
15	marked left esotropia	+ 20	15	+	+	+
16	very small right hypotropia		00	+	+	-
17	very small left esotropia	+ 1	54	-	-	-
18	very small right esotropia	+ 5°	15	+	+	+
19	very small right esotropia	- 2	50	±	±	+
20	very small left esotropia	+ 20	09	±	±	-
21	very small right esotropia	+ 5°	00	+	+	+
22	very small right esotropia	+ 4	43	+	±	+

+ asymmetrical

- symmetrical

± presumably asymmetrical

• a patient with anisocoria

Table II  
Anisometropia (straight eyed cases)

Anisometropia	0-1.4	1.5-2.9	≥ 3.0
Asymmetrical fundus reflexes	9	0	2
Symmetrical fundus reflexes	83	5	0

The sensitivity and specificity of the method for anisometropia of 3.0 diopters or more  
 $\alpha$  = probability of false positive  
 $\beta$  = probability of false negative

$$\text{sensitivity} = 1 - \beta = 1 - \frac{0}{5 + 0} = 1.00$$

$$\text{specificity} = 1 - \alpha = 1 - \frac{9 + 2}{(9 + 2) + (83 + 5)} = 0.89$$

The anisometropia was 1.5-2.9 diopters in seven cases of which two asymmetrical and five symmetrical fundus reflexes

The anisometropia was 3.0 diopters or more in five cases 3.0 3.1 3.1 4.1 and 8.9 diopters. All of these had asymmetrical fundus reflexes. The probability that this result is dependent on a change is less than 0.01 ( $p < 0.01$ ).

### Hyperopia (Table III)

(The deviating eyes are excluded from this material)

The hyperopia was under +4.5 diopters in 93 eyes in none of them a light crescent seen in the fundus reflex

The refraction was between +4.5 and +6.0 diopters in eight eyes in three of which a light crescent appeared in the low part of the fundus reflex. For five eyes which did not have crescents in the fundus reflex the pupils than those with pupillary crescents

There were no refractions of over +6.0 diopters among the straight and deviating eyes were not included in this hyperopia material. It should be mentioned that the only great hyperopias, about +1.5 diopters, were in deviating eyes in each of which a marked hyperopic low part fundus reflex crescent appeared.

Table III  
Hyperopia (straight eyed cases)

Refraction	$< +4.0$	$\geq +4.5$
Eyes with low part pupillary crescent	0	3
Eyes without low part pupillary crescent	90	2

a (Table IV)

deviating eyes are excluded also from this material)

refraction was less than  $-2.0$  diopters myopia in 19 eyes of which only had a small crescent in the upper part of the pupil. The refraction in these eyes was  $-2.0$  comb cyl  $+0.5$  ax 0. The pupillary crescent was absent in 18 myopias.

myopia was from  $-2.0$  to  $-2.9$  diopters in 19 eyes of which two eyes had an upper part pupillary crescent and 17 did not.

myopia was from  $-3.0$  to  $-3.9$  diopters in 16 eyes. Nine of these had an upper part pupillary crescent and seven did not.

refraction was from  $-4.0$  to  $-4.9$  diopters in four eyes. Three had upper part pupillary crescents and one in which the refraction was  $-4.0$  did not.

myopia was over  $-4.0$  diopters in 14 eyes (mean  $-5.5$  diopters) all with upper part pupillary crescents. This result is statistically very significant  $P < 0.001$  ( $\chi^2$  test d.f. = 1). The size of the crescents was roughly proportional to the amount of the myopia but a very small difference in crescent size was seen in eyes with the same amount of myopia.

no crescents of any kind appeared in association with refractions of less than  $-1.75$  diopters myopia or less than  $+4.5$  diopters hyperopia and the total number of eyes within this range was 172.

astigmatism

there was over two diopters astigmatism in 11 eyes. It was combined with either hyperopia or myopia as direct astigmatism in all the cases. It was not detectable from any changes in the photographs. The same was true of the other astigmatism in 90 eyes.



anisometropia of 3.4 diopters which nevertheless had symmetrical fundus reflexes. This patient had an anisocoria that can be explained as the reason for the symmetrical fundus reflexes. The pupil of the deviating and more hyperopic eye would be lighter as expected by suitably shaded by the smaller pupil on the same side. Very close examination of the photograph afterwards showed some lightness beside the pupil in the low part of the fundus reflex of the deviating eye which supports the explanation. This small change was not noted by either person examining the photographs.

In hyperopia of +4.5 to 6.0 diopters the light crescent appeared in the upper part of the pupil only in three eyes and did not appear in five. This method is test as such unreliable for detecting hyperopia of this amount. Large hyperopias were only seen in the deviating eyes in the material and in these cases hyperopic crescents appeared. The patients were evidently accommodated when they fixed their gaze on the small signal light of the camera and therefore the hyperopic crescents did not appear in the quantities that might have been expected in comparison with the results of the demonstration eye in the previous article. Further photographic examinations in cycloplegia should be made in hyperopic cases in order to obtain more information on this point.

In myopia the crescent appeared in the upper part of the pupil in 14 eyes between -1.75 and -4.0 diopters and in seven eyes between -4.0 and -6.0 diopters (14 eyes average -3.5 diopters). This result is statistically very significant with chi square testing ( $P < 0.001$  d.f. = 1). Thus the method is quite reliable for screening high myopias without cycloplegia.

The variable size of the pupils apart from accommodation is one of the reasons for the variable appearance of the hyperopic and myopic crescents in the same refraction values. Therefore photography in cycloplegia should be tested for more accurate screening of refractive errors.

Astigmatism did not cause any specific changes in the photographs. This is natural because the method measures the refraction roughly in the vertical meridian. The horizontal meridian could also be measured by turning the camera source 90° which would require an extra photograph. This would make it possible to estimate the presence of astigmatism from the different appearances of the fundus reflexes in the same eye photographed in the separate meridians according to the principle of static skiascopy. Further experiments however are needed in this respect.

The present study gives a favourable view of this type of photographic method for the objective screening of strabismus in children. Large strabismic anisometropias and myopias can also be found. In screening hyper-

ologic medication should be used. This anyway often changes the natural strabismus situation of the patient.

Worthy of consideration is the fact that the manifest leukokorias and lesions in transparent ocular media can also be discovered with the method, since they cause noticeable changes in the fundus reflex photographs. The photographs have standard scale. Therefore follow up measurement of the corneal diameters and other visible eye and face dimensions are possible with the method without touching a child.

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# THE COLOUR RECEPTORS IN AMBLYOPIA INVESTIGATED BY SPECIFIC QUANTITATIVE PERIMETRY

BY

EGILL HANSEN

With a method combining static colour perimetry and the two-threshold technique of Stiles characteristic sensitivities of the cone mechanisms have been studied in amblyopic and non amblyopic eyes. For the red and green cone mechanisms a central depression of sensitivity was found in the amblyopic eye the depression was more pronounced the higher the degree of amblyopia. For the blue cone mechanism no significant difference was found between the amblyopic and non amblyopic eye except at the fixation point where the mean threshold sensitivity was significantly higher in the amblyopic eye than in the leading eye. By registration of blue receptor curves a distinct age factor was observed. In the younger patients (aged 10-16) the sensitivity level at peripheral positions for both the amblyopic and the non amblyopic eye was significantly higher than in the older patients (aged 39-50). This difference is evidently due to the higher absorption of blue light in the ocular media in older patients.

In our cases with eccentric fixation the true fovea represents the relatively best sensitivity of the red as well as of the green cone mechanism.

*Key words:* amblyopia - static colour perimetry - Stiles functions and differences

Static perimetry has proved to be a suitable method for studying the functional defects in amblyopia (Mackensen 1959, François et al 1965). By static perimetry under ordinary conditions no differentiation can be made between the red and green cone mechanisms (Sloan 1950) nor between the individual cone mechanisms.

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ation of different cone systems can be obtained by the method of  
e chromatic adaptation combined with static perimetry in the Gold  
perimeter (Hansen 1974) It is the purpose of the present work to report  
characteristic findings in cases of amblyopia by use of this method

### Material

e patients 10-59 years of age were examined The principle facts are  
in Table I There were 4 males and 8 females 5 had strabismic ambly  
nd 4 had anisometropic amblyopia Mild amblyopia (visual acuity better  
/20) was found in 5 patients medium amblyopia (visual acuity 6/20) in  
and severe amblyopia (visual acuity 6/60 or less) in 6 patients All the  
ts had normal visual acuity (6/6) in their best eye except patient F in  
the leading eye had been lost by injury one year previously Four  
ts had eccentric fixation in their amblyopic eye and 8 had central  
n. Ophthalmological examination was otherwise normal in all patients

*Table I*

Twelve amblyopic patients examined by specific quantitative perimetry

Age and sex	Type of amblyopia	Visual acuity	Fixation
39 M	Anisom	6/60	Central
49 M	Strab	2/60	Grossly periph Nasal
50 F	Strab	3/60	Parafov Nasal
16 F	Anisom	6/60	Central
27 F	Anisom	6/10	Central
24 M	Anisom	5/60	Parafov Nasal
59 M	Anisom	6/12	Central
10 F	Strab	6/20	Central unstable
42 F	Anisom	6/12	Central
26 F	Strab	< 1/60	Periph Nasal
11 F	Strab	6/19	Central unstable
11 F	Anisom	6/20	Central

## Methods

The type of fixation was determined by means of a visuscope Colson 1974. The patients were examined with the Ishihara's test (11th edition) and the AO-HRA test (1974) and in some patients with the Larnsworth's D-15 test. Static perimetry and chromatic adaption was performed with a method which has been described earlier (Hansen 1974; Hansen & Seim 1978). Narrow banded interference filters (half band width 10–15 nm) were used. As the targets were elliptical their sizes were indicated as the angular diameter of a circle comprising the same area. The standard size of the target was 54 (object 1).

The patients used correction glasses for the test distance during the examination. If correction was only necessary in the amblyopic eye neutral glasses were used in the other eye. The examinations were performed in a random order for the amblyopic and the non amblyopic eye.

## Results

Static perimetry curves for 2 patients obtained in the standard white illumination of the perimeter is shown in Fig. 1. For one patient (F) the perimetric curve obtained under scotopic conditions is also shown. Patient D with a central fixation still had some asymmetry of his perimetric profile. Patient F with parafoveal nasal fixation showed a displacement of the blind spot and the peak sensitivity towards the nasal side. By scotopic registration a similar displacement though more irregular was seen.

By central registration against blue and purple background the spectral sensitivity curves show the characteristic pattern of the red and green cone mechanisms (Fig. 2). The response curve of the amblyopic eye is markedly reduced but otherwise identical with that of the non amblyopic eye.

By registration of spectral sensitivity against a yellow background the curves obtained in patient C were almost identical for the amblyopic and the non amblyopic eye the peak sensitivity being at about 439–451 nm which corresponds to that of the blue mechanism. The registration here was at a low position. Object lights corresponding to the maximum sensitivity of the cone mechanisms have been used for the perimetric registrations in the following and are indicated in Figure 2 by vertical lines.

Static perimetry performed in a blue background light is shown in Fig. 3. There is marked difference between the normal and the amblyopic eye in patients with severe amblyopia. The curves of the amblyopic eye are depressed in the central part.

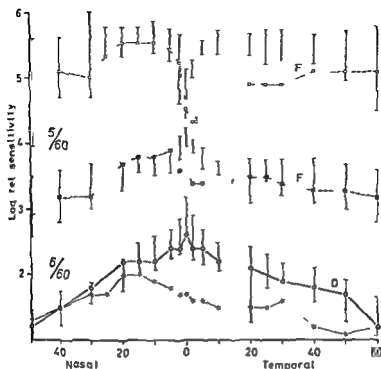


Fig 1

perimetry during dark adaptation (top curve) and in white standard illumination ( $d/m^2$ ) performed in the only amblyopic eye of patient F and in both eyes of D (lower curves). A green target ( $\lambda = 510 \text{ nm}$ ) was used for registrations in F and 19 and 24 white targets for the registrations shown by the middle and the bottom curves respectively. Dotted lines indicate the amblyopic eye for which the visual rates are indicated. The middle curve is displaced vertically by 1 log unit. The bars indicate the normal variation ( $\text{mean} \pm 2 \text{ SD}$ ).

ent F with an eccentric fixation has maximum sensitivity 2-3 to the side.

ic perimetry curves obtained during adaptation to purple light are shown. 4 There is a general depression of the sensitivity level of the amblyopic in the same way as was found for the red receptor mechanism. However differences here are less pronounced. Patient C with parafoveal nasal fix shows a maximum sensitivity of the amblyopic eye at 5 to the nasal in a manner similar to patient F.

shows the perimetric profiles of 8 patients registered in yellow light a blue violet target. There are some irregularities especially towards the

periphery the sensitivity level of the amblyopic eye being partly even partly below that of the leading eye. Only patients F and J show asymmetry expressing their eccentric fixation and in patient J a large scotoma was indicated near the fixation point. Patient C apparently also had eccentric fixation with his amblyopic eye during adaptation to the yellow light.

A characteristic feature appears from the perimetric profiles the mean sensitivity of the blue mechanism is not represented by the fovea but by a parafoveal region. There is a distinct foveal dip in the perimetric sensitivity curve. For 7 patients with a central fixation of both eyes (A, C, D, H, I, K, L) the mean differences of threshold sensitivity found in the normal and amblyopic eye at different perimetric angles are shown in table II. The

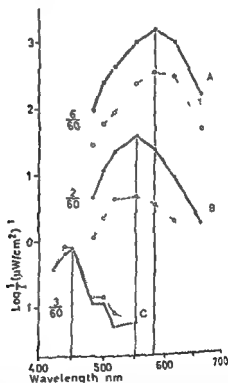


Fig. 2

Spectral threshold sensitivity registered against a blue background (A/Wratten 34 A 2000 lux), a purple background (B/Wratten 34 A 2000 lux) and a yellow background (C/Wratten 34 A 2000 lux). The registration is central (A, B) and nasal (C). The curves for patient A are displaced vertically by  $2\frac{1}{2}$  log units and those of patient B by three log units. Dotted lines indicate the amblyopic eye, shaded area the normal variation. 1 sd. Angular size of target is  $4'$ .

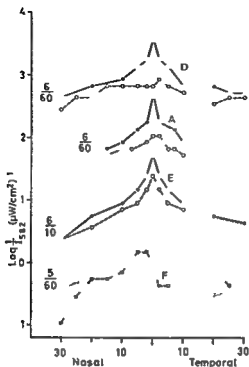


Fig 5

perimetry against a blue background (Watten 4/ 165 lux) Target subtending angular size  $\lambda_m = 532$  nm Absolute threshold sensitivity is indicated correctly for each graph those of the other patients are displaced vertically and separated by 1 Full line indicates the leading eye and stippled line the amblyopic eye The visual rates of the amblyopic eyes are indicated The shaded area indicates the normal variation (mean  $\pm 1$  SD)

1 predominance of one eye over the other Only at the fixation point a significant difference which is in favour of the amblyopic eye being higher than in the non amblyopic eye ( $P < 0.01$ )

Another characteristic feature is the relation of the age factor to the sensitivity of the blue mechanism The curves to the right in Figure 5 represent younger patients and are generally at a higher sensitivity level than the curves to the left representing the older patients In table III comprising 7 patients the mean threshold sensitivity of the older patients (39-50 years) at different perimetric angles are compared with those of the younger patients (16 years) The values of the amblyopic and the non amblyopic eye were



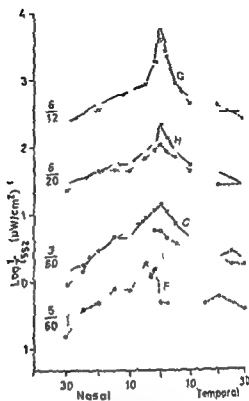


Fig. 4

Static perimetry against a purple background (Wratten 31 A 900 lux) Target size angular size  $\lambda_{\text{max}} = 552 \text{ nm}$  Explanation as for Fig. 3

calculated together. At 30° nasal position only inconstant response to the target was recorded in the older group while good sensitivity was indicated in the younger group. Significantly higher mean values were found in the younger group at the perimetric angles 20° and 10° at the nasal side and 20° at the temporal side.

### Discussion

Contrary to what is expected determination of increment thresholds in the amblyopic eye was often more sharply indicated than in the non-amblyopic eye. This is in accord with experience with partially sighted persons who are more often able to indicate their thresholds sharply when judging brightness contrasts than normally sighted persons (Lie 1974).

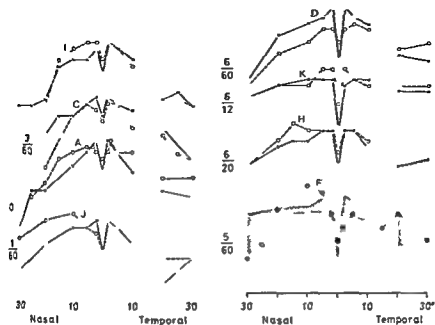


Fig 5

perimetry against a yellow background ( $\lambda = 589 \text{ nm}$  7000 lux) Target subtending 54 angular size  $\lambda_m = 451 \text{ nm}$  Explanation as for Fig 3

Table II

differences of threshold sensitivity for the normal and the amblyopic eye ( $S_N - S_A$ ) in patients registered with a blue target against a yellow adapting background  
SD = standard deviation

Perimetric angle	$S_N - S_A$ (Log units)	SD	Significance t	P
90°	0.19	0.29	1.72	
10	-0.01	0.19	0.13	
5°	-0.12	0.19	1.66	$P > 0.1$
2	0.02	0.23	0.23	
0	-0.21	0.15	3.76	$P < 0.01$
5°	0.04	0.20	0.49	
10	0.03	0.05	1.55	
20	0.03	0.24	0.89	$P > 0.1$
30°	0.07	0.21	0.26	
30°	-0.01	0.29	0.12	

Table III

Differences of threshold sensitivity in two groups of patients registered with a target against a yellow adapting background. Sensitivity is calculated as  $L_{50}^{-1}$  ( $\mu W/cm^2$ )<sup>-1</sup>.  $I$  = absolute irradiance from test spot so standard deviation

	Peri- metric angle	Mean sensitivity		Differ- ence	Common SD	Significance	
		Group I (39-50 N=3)	Group II (10-16 N=4)			t	P
Nasal	30°	inconstant	0.40				
	20°	-0.33	0.49	0.87	0.95	4.60	P<0.01
	10°	0.22	0.62	0.40	0.16	3.38	P<0.01
	5°	0.35	0.69	0.34	0.19	2.99	0.05 < P < 0.1
	2°	0.40	0.71	0.31	0.19	2.19	
	0°	0.08	0.96	0.18	0.34	0.0	P>0.1
Temporal	2°	0.43	0.70	0.27	0.17	2.11	0.05 < P < 0.1
	5°	0.35	0.63	0.28	0.23	1.59	P>0.1
	10°	0.96	0.58	0.32	0.29	1.47	
	20°	-0.06	0.32	0.38	0.16	2.99	P<0.01
	30°	-0.43	0.37	0.80	0.29	3.64	P<0.01

It is considered that the primary defect in amblyopic eyes lies in the perception mediated by cones in the central area. For the red and green cone systems the results obtained in our patients show that in both cone systems there is a generalized depression of sensitivity in the amblyopic eye and this is more pronounced the higher the degree of amblyopia. The same kind of generalized depression of sensitivity is found by ordinary static perimetry (Fraunhofer 1968) and by registration of increment threshold spectral sensitivity (Zaretsky and Szucs 1956, Harwerth and Levi 1977). On the other hand, Wald and Berman (1914) found the light thresholds of amblyopic eyes to be essentially normal (using 1-2 targets). By registration of the red as well as the green cone responses (Fig 3 and 4) in eyes with eccentric fixation and severe amblyopia there is a characteristic displacement of the peak sensitivity to temporal and nasal periphery from the fovea. The sensitivity at this point is only moderately reduced. On the contrary in patients with central fixation and severe amblyopia (A. D. Levi 1977) there is a considerable depression of the foveal sensitivity. This is confirmed by the conclusions by Mackensen (1959) using registrations in white light that the foveal sensitivity in amblyopic eyes is relatively high when the fixation is eccentric while it is much depressed when the fixation is central.

different response pattern was found for the blue receptor mechanism a significantly higher foveal sensitivity was found in the *amblyopic* eye in the leading eye. This difference might be due to instability of fixation or the same difference of foveal sensitivity is found also in patients with a stable central fixation. Evidence of greater receptive fields in amblyopic has been found by Flynn (1961), Lawwill et al (1973) and others. The foveal sensitivity of the blue mechanism in amblyopic eyes may possibly be ascribed to differences in receptive fields implying also mechanisms of lateral inhibition. A better threshold sensitivity to short wavelength stimuli to long wavelength stimuli in amblyopic eyes has also been reported by Land and Szuchs (1956) and by Israel and Verriest (1972).

It is interesting that patient C with an eccentric fixation apparently had a central fixation after adaptation to the bright yellow light. In cases where the fixation is not too fixed it is likely that a pure longwave illumination stimulates the foveal fixation. It is therefore possible that the Na light of wavelength 589 nm is favourable for stimulating central fixation in cases of stable fixation. Supporting this are the results obtained by Brinker and Katz (1973) in patients with gross eccentric fixation where successful central fixation was obtained after using red filters.

The age difference found in our patients by registration of the blue receptor response may be explained by the higher absorption of the lens in the short wavelength range of the spectrum (Weale 1954). That the difference in sensitivity to blue targets is most pronounced in the periphery is consistent with a pure lens absorption. Yokoyama et al (1978) found differences in the response of the blue mechanism by ERG in phakic eyes according to age but in aphakic eyes the response of the blue cone system was almost the same regardless of age.

The quality of colour vision has often been found by a higher degree of blue blindness in amblyopia where it resembles that observed in normal eyes at eccentric positions (Land and Verriest 1967). Normal colour vision was found in all our patients by binocular examination. Some discrepancy in the cases of severe amblyopia may be ascribed to the reduced sense of form which may greatly influence the reading of pseudo isochromatic charts (Hansen 1963). Characteristically one patient (A) was not able to read the figures on the Ishihara charts with his amblyopic eye though he could clearly indicate the colours. By eccentric fixation Roth (1968) pointed out that colour deviation in amblyopia is the normal colour sense of the fixation area and that the colour sense of the fovea remains normal even when the fixation is extra foveolar. Our finding of maximum sensitivity of the red and green mechanisms corresponding to the displacement of the fovea supports this.

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## CLINICAL ELECTRO OCULOGRAPHY

BY

A. PINCKERS

In clinical routine EOG we are dealing with a base line or lightsensitive potential and a lightsensitive potential the latter consisting of at least two different oscillations a fast negative and a slow positive one. Any judgement of EOG without referring to the absolute level of the baseline is an incomplete one. It is no longer justified to refer to the Arden ratio as a unique parameter for EOG normality or abnormality. Our knowledge about the generating mechanisms of the different EOG components is insufficient. Clinical EOG examination is one way to better understanding. Statistical evaluation is not easy because of the considerable inter- and intraindividual variations. The study of unocular diseases or affections may solve some of our problems.

*Key words:* electro-oculogram - slow oscillation - fast oscillation - base line - Arden ratio - A-criterion

In ophthalmic literature the clinical electro-oculogram is usually expressed as a quotient the lightpeak/darktrough ratio (Lp/Dt) of Arden. The Lp/Dt ratio reflects an aspect of the EOG the so-called slow oscillation or lightpeak. The Arden ratio is used to classify a routine clinical EOG as normal, subnormal or disturbed. An EOG with a normal Lp/Dt ratio can be definitely abnormal because there is a pathologically lowered base line. The purpose of this study was to evaluate routine clinical EOG recordings at the Nijmegen Ophthalmic Clinic in order to get an impression of the possibilities of EOG examinations. The results of this analysis are discussed in the present paper.

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## Materials and Methods

The material for this study consists of about 3000 patients examined under clinical conditions. The EOG examination is the same as described in previous studies (Pinckers & Thijssen 1969, 1974; Thijssen et al 1974).

The Initial value ( $I_v$ ) represents the first registration. The Dark trough ( $D_t$ ) is the mean of the measurements at the 6th, 8th, 10th and 12th min. The Light peak ( $L_p$ ) is the mean of the 4 maximum values during light adaptation.  $L_p - D_t$  presents the  $L_p$  is the mean of the registrations between the 7th and 11th min. at the beginning of the light adaptation. In every EOG we calculate the A-criterion ratio and the A-criterion:  $A = L_p - (0.61 \times I_v + 0.91 \times D_t)$  where  $A$  is the intercept of the regression plane on the  $L_p$  axis for normal eyes (Table I; Thijssen 1969).

## Normal values

The group of patients comprises also normal patients for example during control examinations. As with the other patients these normal patients as a rule are familiar with the examination procedure. This is a kind of continuous control for the standardization of the technique and at the same time an ideal group which can be compared with the other patients. There was no significant difference between the 80 normal eyes and the original 76 eyes who served for the standardization of the EOG technique (Pinckers & Thijssen 1969). I therefore decided to consider the normal patients as control group (Table I).

As published elsewhere we found no systematic differences between the right and left eye (Pinckers & Thijssen 1969). The results of right and left eyes therefore can be taken together in calculations. There are however unocular diseases and consequently calculated right-left differences with regard to the  $I_v$  and the  $L_p$ . The right-left difference is expressed as the percentage of the highest value. For example  $I_vRE = 550 \mu V$ ,  $I_vLE = 520 \mu V$ ,  $I_v \text{ diff} = \frac{550-520}{550} \times 100\% = 5\%$ .

Table I  
Normal FOC values (90 eyes, 40 normal patients)

	$I_v$	$D_t$	$L_p$	$L_p - D_t$	A-criterion
mean ( $\mu V$ )	502	389	86	9.51	+
standard deviation ( $\mu V$ )	176	143	29.5	0.49	+
mean + 2 SD ( $\mu V$ )	250	175	437	1.59	+
lower 5% limit ( $\mu V$ )	250	193	430	1.90	+
lowest value ( $\mu V$ )	150	112	325	1.63	+

Iv diff was 13.8%. In 40% of the normal patients Iv diff < 10% in 10% was 10% and in 50% Iv diff was more than 10%. The mean Lp/Dt diff was In 90% of the cases Lp/Dt diff was less than 20% in 87.5% less than 15% less than 10% and in 42.5% less than 5%.

tive potential

sensitive component of the EOG consists of at least two components a with a culmination time of approximately 9 min and a fast oscillation culmination time of 1 to 2 min (Kolder & North 1972).

atio and A-criterion

oscillation essentially represents the light rise or light peak (Lp). It is thus used in the form of the Lp/Dt ratio as the criterion for clinical EOG. As there is no sharp delineation between a normal and a definitely abnormal Lp/Dt. In order to obtain a better separation between normals and abnormals we used the A-criterion in 1969. The A-criterion is calculated on the basis of values (Lp, Dt and Lp) the Arden ratio on two values (Dt and Lp). Since 1968 we used both the Arden ratio and the A-criterion. Table II gives the results of examination of 512 consecutive recordings. From these I selected cases in which we expect a normal EOG, for example in congenital functional anomalies, albinism, carriers of ocular albinism.

As a 90% limit the Arden ratio (91.4%) is better than the A-criterion (71.6%). It is clear that the Iv is responsible for this difference. The Iv reflects the pre-blink period. In a darkadapted situation the corneoretinal standing potential

Table II

ratio and A-criterion in 512 consecutive EOG recordings. Out of these 81 recordings on the basis of the clinical diagnosis were expected to be normal.

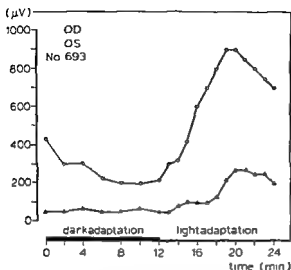
	No	Lp/Dt ≥ 180	150 ≤ Lp/ Dt < 180	Lp/Dt < 150	A ≥ +83	0 ≤ A A < +83	A < 0
normal	81	74	7	0	58	23	0
norm (%)	100	91.4	8.6	0	71.6	18.4	0
in	512	293	91	198	172	103	237
(%)	100	43.6	17.8	38.7	33.6	20.1	46.3



**Table II**  
Some examples of selective impairment of a single FOC mechanism

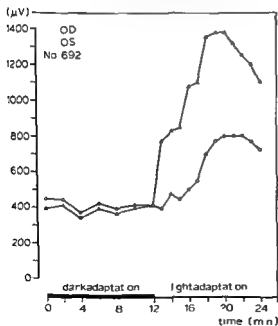
Base line involvement								
Case No	diagnosis	eye	Iv	Dt	Lp	lp Dt	lp Dt d/f	5 min d
693	normal	RE	400	900	900	450		
	perf injury	LE	50	50	900	540	81	1
985	normal	RF	400	380	1000	960		
	perf injury	LE	80	80	400	300	81	1
4433	normal	RE	500	450	1000	960		
	perf injury	LF	200	200	500	950	80	1
5358	choroid effusion	RE	200	200	539	950	80	1
	normal	LE	500	413	999	950		

Lightpeak involvement								
Case No	diagnosis	eye	Iv	Dt	Lp	lp Dt	lp Dt d/f	5 min d
699	siderosis	RE	450	429	797	100	41	<
	normal	LE	400	400	1306	350		
974	normal	RF	400	36	800	43		
	macular disease	LF	350	387	591	150	54	<
1742	siderosis	RF	400	350	600	100	89	<
	normal	LF	400	400	1000	750		
1830	central a. occl	RE	1000	800	1000	120	51	<
	normal	LE	1000	800	1550	100		



*Fig 3*

impairment of the base line OS by perforating injury. Normal Lp/Dt ratio of the left eye although the absolute value of the lightpeak is diminished.  
See also Table IV case No 693



*Fig 4*

the impairment of the lightpeak OD in siderosis oculi. The base line is not affected.  
See also Table IV case No 692

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## EFFECT OF OILY VEHICLE ON OCULAR PILOCARPINE CONCENTRATION

BY

LOTTA SALMINEN

The effect of 1.4% polyvinyl alcohol (PVA) vehicle and of castor oil vehicle on ocular pilocarpine concentration was studied by radioactive method in the rabbit eye. Statistically higher radioactivities were measured from the anterior surface structures of the eyes dropped with oily vehicle when compared to PVA vehicle at 120 min. It is concluded that the conjunctiva and the cornea serve as a drug reservoir for the longlasting drug effect observed in the literature after oily pilocarpine drops.

**Key word:** rabbit eye — pilocarpine concentration — vehicle — polyvinyl alcohol (PVA) — castor oil

*in vivo* effect of pilocarpine on the pupil in an oily solution is of greater degree and duration than the effect of the same amount of pilocarpine given in methylucose (Borgmann & Wuster 1973), polyvinyl alcohol (PVA) (Saari et al. 1978a) or aqueous (Smith et al. 1978) vehicles. Also oily drops of pilocarpine induce lower intraocular pressure and smaller maximum diurnal variation than PVA drops of the drug (Saari et al. 1978b).

In the present study the greater ocular penetrability of pilocarpine in oily vehicle is demonstrated when compared to the same amount of drug in 1.4% PVA vehicle.

### Material and Methods

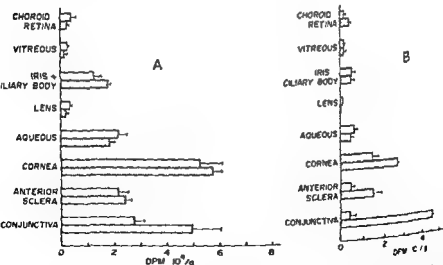
Seventy adult albino rabbits weighing 2.5 to 3 kg were placed in individual animal cages where they remained during the experiment. Twenty µl of a solution of labelled and unlabelled pilocarpine was instilled onto the upper part of the cornea.

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During the instillation the upper lid was pulled slightly away from the globe and 120 min after the instillation of the drug the rabbits six in each group were killed by a rapid intravenous injection of pentobarbital sodium (Nembutal®). The eyes were immediately enucleated, gently rinsed in running water and frozen in liquid nitrogen. The dissection of the frozen eyes and liquid scintillation counter samples were carried out as described previously (Salminen 1976).

The tracer drug tritiated pilocarpine in alcoholic solution (41 Ci/mmol) and obtained from the New England Nuclear Corporation, was evaporated to dryness under vacuum. The tracer material was dissolved in 14.7 ml with 2% pilocarpine hydrochloride and in castor oil vehicle with pilocarpine. The PVA solution contained 0.004% benzalkonium chloride whereas the castor oil solution contained no preservative. The final solution radioactivity of  $111 \times 10^4$  disintegrations per min per microliter of vehicle.

The results are expressed in terms of radioactivity, that is the non-radioactive material and the bioactive tritiated pilocarpine are ignored in the calculations. The carrier substances were not chemically identical in the two vehicles and the radioactive material was not re-examined for its bioactivity during the study. However, extensive metabolism of the labelled material during the study is unlikely (Chrai & Robinson 1974; Lazare & Hottington 1975). Thus the measured radioactivity can be considered as a measure of the effect of the two vehicles on pilocarpine penetration into the eye.



*Fig. 1*

Ocular radioactivity at 60 min (A) and at 120 min (B) after instillation of 10 µl of tritiated pilocarpine (41 Ci/mmol) in 20 µl of 1.4% polyvinyl alcohol (PVA) (open columns) and 20 µl castor oil (shared columns). Carrier substance in PVA 99% pilocarpine hydrochloride and in castor oil 2% pilocarpine 1 × 10<sup>4</sup>.

## Results

Radioactivities obtained by liquid scintillation counting from various ocular structures are presented in Fig. 1. At 60 min radioactivity in the conjunctiva was significantly higher ( $P < 0.01$ ) after castor oil vehicle when compared with 14% vehicle, whereas at 120 min radioactivity in all anterior surface structures was significantly higher ( $P < 0.001$  or  $P < 0.01$ ) in eyes with oil vehicle. Low radioactivities were measured in all parts of the inner coat of the eyeball.

## Discussion

These results suggest a longer ocular surface contact time of drugs dissolved in oil vehicle and it is in agreement with the microscintigraphic study on petrolatum and oil ointment by Hardberger et al. (1975). Because the enucleated eyes were stored in water no information of the drug and vehicle in the conjunctival cul-de-sac mixed with the tear fluid has been obtained.

The longer surface contact time after oil vehicle led to a greater drug penetration in the conjunctiva and cornea. Drug in the conjunctiva and cornea obviously served as a drug reservoir even after the wash-out of the drug and vehicle mixed with tear fluid and contributed to the longlasting pilocarpine effect of oil drops.

The development of soluble drug vehicles for first-order kinetic systems is a great challenge for the medical industry. Optimal drug therapy of the eye could thus be achieved with lesser drug induced side effects, with smaller drug doses and with fewer applications.

## Acknowledgments

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# INFANTILE GLAUCOMA ELECTROPHYSIOLOGY

BY

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Thirty-eight children with congenital infantile glaucoma (hydrophthalmia) were examined using electrophysiological methods. In contrast with adult glaucoma, hydrophthalmia is characterized by early changes in the electroretinogram and rise of the threshold of electrical phosphene, which points to serious pathological changes in the retinal optic nerve. In such cases surgical intervention is indicated.

**Keywords:** infantile glaucoma — electroretinogram — electrical phosphene

State of visual functions in children with congenital glaucoma (hydrophthalmia) has been comparatively poorly studied, partly because of the difficulties caused by functional examination of children. In particular, little information is available on objective clinicophysiological examination (electroretinogram, ERG, visual evoked potential, EOG, and electroencephalogram, EEG). Francois et al (1956) examined 91 patients with congenital glaucoma and noted in most of them changes in visual acuity, field and adaptation. These changes, however, were not specific to hydrophthalmia but could also be observed in other forms of glaucoma. On the other hand, some other changes were discovered in the electroretinogram of nearly all patients (decrease in the amplitude of b-wave and other), not characteristic of open angle glaucoma of adults. Some increase was noted in the basal values of EOG but the EEG remained unchanged. Changes in the ERG of patients with infantile glaucoma were also observed by Pagani (1959) (cited after Jayle et al 1960). Characteristics of the phasic development of infantile glaucoma are usually based on

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cliniomorphological data and practically do not take into account the functional state (Lagrange 1925, Cherkasov 1963, Kovalchuk 1971).

In the present investigation it was attempted to establish a relationship between the stages of the periodization derived from cliniomorphological data and the functional parameters, in particular with the ERG and electrical sensitivity of the eye.

## Methods

Ocular examination included examination of the anterior segment of the eye (oblique) illumination, measurement of cornea diameter with a keratometer and biomicroscopy (tonometry, tonography).

Small children were examined (including measurement of intraocular pressure) under anaesthesia (deep premedication). Visual acuity in children above 3 years was determined by current methods. Determination of visual acuity in younger children were possible. In children above 5-6 years the field of vision was examined on a projection perimeter.

The ERG was recorded on a commercial electroencephalograph with a projection system. New methods of functional diagnostics and ophthalmology (Moscow 1971) and the diagnostic significance of electrophysiological indices in the major diseases of the visual neural apparatus (Moscow 1976). For the leading off of biopotential silver electrodes were used mounted into a contact lens. They were fitted in each individual case from a special device for the examination of children. The flash energy of the light stimulus was 1-5 joules, duration 30  $\mu$ sec, visual angle of the stimulus 30°, distance from the patient to the electrode 10 cm. Both single and rhythmic stimuli were presented. Very young and non-cooperative children were examined under conditions of profound premedication (nembutal, 0.05-0.1 mg/kg, diphenylhydramine hydrochloride, chloral hydrate) in doses appropriate to the age of the patient (Kovalchuk 1971a, b). Examination began after a 40-min sleep period under laboratory illumination. After the placement of electrodes the children remained in darkness for 10 min. No mydriatics were used. The children were examined in a position in a light and electrical screened cabin. All the electroretinograms were performed on a natural pupil. Patients undergoing miotic therapy were examined the evening before and the day of the examination and their pupils were dilated noticeably from that of glaucomatous and healthy eyes.

In older children (from 7 years and above) electrical sensitivity of the eye was investigated. i.e. we were able to determine the threshold of electrical phosphene by the technique developed at the laboratory of physiological optics (Bogdanov 1971) with the aid of the electrostimulator "TEST-1" designed by the experimental unit of the USSR Academy of Medical Sciences. The threshold of electrical phosphene was determined by the method of constant stimuli. From minimum current intensity at which the eyeball evokes a luminous sensation (electrical phosphene) the frequency of stimulation was also examined. It is characterized by the highest frequency of stimulation at which the threshold intensity at which the sensation of flickering disappears. The threshold of electrical phosphene is a response to the electrical stimulus of the neurons of the inner nuclear layer and fibres of the optic nerve (Bogdanov 1971, Bogdanov and others 1971, 1973). It is a response to the stimulus of neurons of the axial bundle of the optic nerve (Bogdanov 1963). The threshold current does not excite the photoreceptors and does not break down the visual purple.

control group consisted of 50 healthy children aged from 1 to 14 years examined in connection with a genetic consultation of families handicapped by hereditary eye diseases (retinitis pigmentosa etc.). As the amplitude of the  $a$  and  $b$ -waves does not change noticeably in the first 2 years of life, it was possible to compare the averaged data obtained for the control group with the parameters of the ERG of patients with different stages of congenital glaucoma.

## Results

Eighty children (67 eyes) with congenital glaucoma (hydrophthalmia) aged from 1 to 14 years were examined using the methods described above. According to the markedness of pathological symptoms five stages of hydrophthalmia can be distinguished. No definite relationship between age and stage of the disease was found. What is more, in one and the same child the stage of the glaucoma was often different in the right and left eye. In most cases (21 children) an already developed glaucoma was diagnosed at the age of 3 to 7 years. Hydrophthalmia was present in initial stages in 11 eyes, developed hydrophthalmia in 25 eyes, advanced in 14 eyes, nearly absolute in 11 eyes and absolute in 6 eyes.

The early stages of the disease included the following findings: an enlarged eyeball, with a corneal diameter not exceeding 12 mm, no dilated limbal vessels, anterior chamber deeper than normal, single ruptures of Descemet's membrane, glaucomatous excavation of the optic disc either absent or in the initial stage, microscopically ascertained embryonal tissue in the angle of the anterior chamber, increased intraocular pressure. In the developed stage the eyeball was markedly enlarged, corneal diameter 13–14 mm, limbal dilated, ruptures of Descemet's membrane, confined corneal opacities, deep anterior chamber, hypoplasia of the embryonal tissue in the angle of the anterior chamber, in many patients marked glaucomatous excavation of the optic disc and a high intraocular pressure.

The symptoms of advanced hydrophthalmia were: greatly enlarged eyeball, corneal diameter above 14 mm, strongly dilated limbal vessels, multiple ruptures of Descemet's membrane, corneal opacities, anterior chamber very deep, embryonal tissue in its angle, high intraocular pressure, visual acuity considerably reduced.

In nearly absolute glaucoma photoperception was preserved with uncertain or incorrect light projection.

In children with absolute glaucoma vision was 0.

Intraocular pressure was up to 27.0 mmHg in 12 eyes, 28–32 in 21 eyes and 33 or more in 34 eyes.

Visual acuity could be measured in 29 children (54 eyes). It was 0.3–0.4 in three, 0.01–0.09 in eight and 0.01–0.09 in 27 eyes. Photoperception with correct light projection was preserved in 10 eyes, 6 eyes were blind. In 9 small children (13 eyes) visual acuity could be not approximately determined.

Table I

Mean parameters of FRC and electrical excitability of visual system in 34 patients with hydrophthalmia

FRC (Empl)		Reproduction of flickering		Electrical excitability	
a-wave $\mu V$	b-wave $\mu V$	White light Hz	Red light Hz	Threshold of electrical sensitivity $\mu V$	Correlation of photoreceptor response
$20 \pm 5.3$	$130 \pm 19.1$	$36 \pm 4.5$	$24 \pm 4.0$	$1.8 \pm 0.8$	1.0

The basic parameters of the ERG (electrical sensitivity and latency in children with hydrophthalmia) are presented in Table I.

A distinct decrease may be noted in the values of the basic components of ERG and electrical excitability. The change in these values with the progression of disease are presented in Table II.

### DISCUSSION

The results obtained revealed a substantial difference between the functional state of the retina in children with hydrophthalmia as compared with that of adult patients with primary glaucoma. The FRC of adults even with absolute glaucoma

Table II  
ERG at different stages of hydrophthalmia parameters

Stage	Indices of the electroretinogram			
	a-wave $\mu V$	b-wave $\mu V$	Rhythm reproduction	
			White light Hz	Red light Hz
Initial	$40 \pm 5.5$	$200 \pm 14.8$	$49 \pm 1.0$	$30 \pm 1.0$
Developed	$32 \pm 1.9$	$160 \pm 8.47$	$43 \pm 1.49$	$20 \pm 1.0$
Advanced	$16 \pm 2.1$	$120 \pm 9.6$	$30 \pm 1.9$	$10 \pm 1.0$
Nearly absolute	$10 \pm 1.8$	$70 \pm 9.5$	$21 \pm 0.9$	$0 \pm 0.0$
Absolute	0 = abs	0 = abs	0 = abs	0 = abs
Control group (healthy)	$54 \pm 5.5$	$270 \pm 9.4$	$50 \pm 4.7$	$30 \pm 1.0$

a rule near to normal in respect to amplitude and frequency (Semenovskaya 1959 Aladjoff et al 1959)

led to discover a connection between the parameters of the ERG electrical sensitivity and liability on the one hand and the level of intraocular pressure on the other. This absence of relationship is probably accounted for by the fact that in hydrophthalmia the degree of functional disturbance of the retina is determined by the stage of the pathological process rather than by the level of intraocular pressure at the moment of examination. It may be assumed that the progressing enlargement of the eyeball with ensuring distension and thinning of the retina are responsible for the disturbance of visual functions. This is confirmed by the data obtained for patients with juvenile and secondary glaucoma. In infantile glaucoma accompanied by eyeball enlargement the parameters of the ERG electrical sensitivity and liability were far less affected by the progression of the pathological process (Khvatova et al 1975). Thus in juvenile glaucoma normal amplitude and frequency parameters were observed occasionally (a wave 50–70  $\mu V$  b-wave 10–20  $\mu V$  rhythm reproduction in white light 50 Hz in red light 44 Hz) when the eyeball was not enlarged. Conversely very low amplitude and frequency values were always obtained with a greatly enlarged eyeball. Thus the a wave was absent in 6 of six eyes out of the fourteen eyes examined in one case it was 30  $\mu V$  and its amplitude value reached 100  $\mu V$ . The b-wave was absent in the ERG of 5 patients with 11 to 30–60  $\mu V$  in 3 patients and 230  $\mu V$  in one patient. A corresponding decrease was observed in the values of rhythm reproduction in white and red light. In patients with secondary glaucoma lingering high intraocular pressure without enlarged eyeball the amplitude and frequency parameters of the ERG and electrical sensitivity were either normal or only slightly reduced (a wave 52  $\mu V$  b-wave 207  $\mu V$  rhythm reproduction in white light 44.1 Hz in red light 33.6 Hz threshold of electrical sensitivity 56  $\mu A$  liability 40.4 Hz).

## Conclusions

The amplitude and frequency parameters of ERG electrical sensitivity and liability are lowered in patients with hydrophthalmia.

The degree of decrease in these parameters rises with the progress of the pathological process from the initial to the absolute stage of congenital glaucoma. During the initial stage the electrophysiological parameters are as a rule normal. With the transition from the initial to the developed and advanced stage to the absolute stage no ERG is elicited electrical sensitivity and liability are greatly reduced (not infrequently no electrical phosphene is evoked).

The character of the ERG electrical sensitivity and liability in hydrophthalmia is due to damage of both the inner and outer layers of the retina apparently

resulting from important structural changes produced in the eyeball by high ocular tension

4 Normal parameters of FRG electrical sensitivity and labile stabilized process when conservative treatment is possible. Changes by reduction of electrical excitability of the visual system are signs of a pathological process requiring early surgical intervention

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# ORBITAL ABSCESS IN TWO NEONATES

## DERIVING FROM CONJUNCTIVAL MALFORMATIONS

BY

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and HANS C. FLEDELIUS

A report is given of two infants who developed an orbital abscess within their first month of life. Generally they were unaffected and malignancies were initially suspected. Ultrasonography showed partly cystic partly solid lesions. The likely infectious foci were conjunctival malformations: a mucosal fistula in case No. 1, a conjunctival cyst in case No. 2.

There was rapid and complete recovery after surgery. The infections were caused by *Staph. aureus* and by *diplococcus pneumoniae* respectively.

Additional findings: Infant No. 1 had a fibroma of the tongue. Infant No. 2 had pes equino-varus.

**Key word:** orbital abscess — orbital tumours — orbital ultrasonography — conjunctival malformations

Orbital infections occur only sporadically in Denmark. The present paper is with two cases affecting neonates where history and clinical findings initially led to a suspicion of orbital malignancy. The two cases are exceptional in that conjunctival malformations appeared as the likely focus of the infections.

### 1

A 4-day-old male infant, number two of two, was admitted to Odense Hospital because of redness and swelling of the tissues of the left orbit.

Family history: Congenital malformations and ophthalmic disorders were not known. In particular manifestations of von Recklinghausens disease could not be disclosed.

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Fig 1

Case No 1 A) prior to surgery: proptosis of the left eye with redness and a 5 mm lid lag B) three days after surgery C) one month after surgery: wound healing in the region. The arrows (A and C) indicate the rounded tumour.

Gestation and delivery were uneventful. Birth weight 3900 g. General physical examination was normal except for a round dense tumour measuring 10 by 10 mm (Fig 1A) at the apex of the tongue.

**Eyes.** Two weeks old he had suffered from a slight conjunctivitis of the left eye for only a few days and treated topically with chloramphenicol.

Three days prior to admission proptosis and redness of the left eye and lid lag with swelling of the ipsilateral eye lids. Chloramphenicol eye drops and nystatin were given immediately, apparently without any effect.

On admission there was a 4-5 mm left lid lag and eye motility was restricted in all directions. The cornea was clear, only slight chemosis, moderate swelling of the eyelids, particularly the lower one, increased retrobulbar resistance. The fundus appeared with slight stasis. The right eye and orbit appeared entirely normal. Repeated examinations of the left conjunctival sac were negative.

Generally the child was unaffected. Rectal temperature was 37.5°C, which decreased to 37.0°C. Heart rate 120/min, count normal but the ESR was high 70 mm/h. Except for the conjunctivitis, no abnormalities were found at oto-rhino-laryngological examination. X-ray examination of the orbits without bone destructions. Maxillary and ethmoidal sinuses were small and without signs of sinusitis.

After two days of systemic treatment with metacillin + streptomycin and chloramphenicol topically without clinical improvement the infant was transferred to a specialist hospital with suspicion of a malignant tumour of the orbit (Fig 1A).

CT scan was inconclusive (technical difficulties due to uncooperative and the small size of the infant) but ultrasound examination revealed a 40 x 30 mm lesion in the retrobulbar space, displacing the diseased orbit on the nasal side of the eye and also behind it (Fig 1B). The lesion was predominantly cystic. Low reflecting structures were seen in the lower part of the lesion. Ultrasound abscess or sarcoma were the most probable diagnoses.

During compression test with the transducer (the eye proved to be less compressible than the eye) a whitish discharge came out in the lower part of the lower fornix through a fistula with smooth mucous walls. There was no further relation to probing of the fistula.

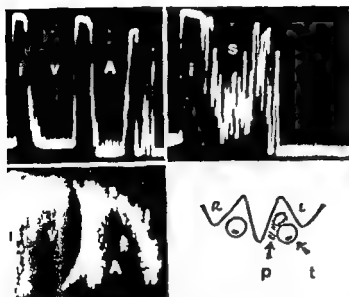


Fig 2

Ultrasonography in case No. 2 Transbulbar examination (marked by t on the schematic fig) shows the eye (i = initial echo v = sound homogeneous vitreous space) before the surgically excised retrobulbar tumour by A-scan (top-left with low reflecting structures abscess cavity) and B-scan (bottom left) Medial parabolar (p) A-scan shows a solid lesion (S) anteriorly in the orbit (top-right)

ical exploration was then performed through the lower lid (cf Fig 1 B) in a plane on the orbital floor. At a depth of about 15 mm an abscess cavity was found. *Cultures* of Staph aureus.

After surgery there was complete recovery and there has been no recurrence. Both eyes appear entirely normal (Fig 1 C) with normal mobility and ophthalmoscopy.

Elementary examinations: Chromosome pattern normal. Urine excretion of glucose amino acids also normal.

## Case 2

A female infant, number two of two, was admitted to Rigshospitalet because of a growing tumour of the left orbit.

History: negative.

Birth and delivery uneventful. Birth weight 3350 g. General physical examination was except for slight pes equino-varus.

Five days after delivery a tumour became visible in the left lower lid. Two weeks later it was described as a 10 × 10 × 10 mm hard nodule covered by unaffected skin.

Soon it had grown to 20 × 15 × 10 mm (Fig 3). It was still dense but reddening skin and small cutaneous phlebectasiae had now developed and the eye was displaced. Fundus appearance was normal in both eyes and the right orbit was normal.





Fig 3

Case No. 2 with displacement of the left eye due to a tumour of the left eye. Transbulbar ultrasonography (t on schematic drawing) shows a tumour (A = abscess) before the eye (V = vitreous space) by A scan (top left) and B (bottom left). On parabulbar examination (p) the abscess cavity is detected (A scan top right B scan bottom right).



Fig 4

(Eye Path No. 186/78 a) Histological section of the conjunctival eye in case 1. L = lumen. Goblet cells staining dark with PAS (arrows) are seen in the nonkeratinized epithelium ( $\times 5$ ).

In the child was unaffected. Haemoglobin normal, ESR 11 mm/h, white blood cell count 19,200/mm<sup>3</sup>, being lymphocytes 15%, neutrophilic granulocytes 75%.

There was no destruction of the facial bones. CT scan showed a soft tissue tumour in the anterior and inferior part of the left orbit.

**Pathology.** A cystic lesion, however, with a solid anterior part, was found in the medial quadrant of the left orbit, located anteriorly, and without evidence of retrobulbar changes (Fig. 3).

Exposure was planned. On incision of the left lower lid, abundant pus appeared, and the lesion could be excised.

Microscopy revealed diplococcus pneumoniae, no fungi.

Pathology (Eye Path. Inst. No. 18678a) showed a conjunctival cyst (Fig. 4) lined by a well-organized multilayered non-keratinizing epithelium with numerous goblet cells. Inflammatory changes were seen in the adjacent stromal tissue and, in one corner of the specimen, lacrimal gland tissue was noticed.

Post-operative course was uneventful. There were no subsequent recurrences.

## Discussion

Orbital tumours in infancy and childhood present many problems concerning early diagnosis and efficient therapy. Therefore, centralization to a multi-specialty team has been advocated in earlier reports (Eldrup-Jørgensen & Fledelius 1975; Fledelius 1976).

The efforts are reflected by the present report, which deals with two neonates who were transferred (case No. 1) or directly admitted (case No. 2) to Rigshospitalet, Copenhagen, due to suspicion of rapidly expanding tumours of the orbit. This diagnostic concept was based on the following features:

Both infants were generally unaffected, especially without fever or other signs of infection. Laboratory tests, however, revealed a markedly elevated ESR in case No. 1 and a slight leucocytosis in case No. 2.

The usual neighbouring sources of orbital infections (cf. Burnard 1959; Esente & Buono 1960; Duke Elder & MacFaul 1974; Jazbi & Rutter 1977) were not involved. In particular, there were no roentgenological changes of facial or cranial bones. The ethmoidal and maxillary sinuses were small and normal, and the lacrimal gland was unaffected.

The lesions presented as localized tumours (Figs. 1 and 3). There were no signs of orbital inflammation (cellulitis phlegmone) which otherwise is to be expected in neonates (Esente & Del Buono 1960; Kuranov 1976) whose defense against offensive microorganisms is incompletely developed.

Ultrasonography yielded concise information about the morphology of presumed tumours showing cyst-like lesions with however without effect of the pressure of the transducer which caused the purulent discharge through the fistula in the lower fornix during diagnostic manipulation (diagnostic ultrasound indeed!)

In both cases the diagnostic problem was finally solved through the intended for biopsy specimens. The offensive microorganisms were *Staphylococcus aureus* and *pneumococcus* respectively.

*Conjunctival malformation were the probable sources of infection in the* In case No. 1 the conjunctival fistula appeared in combination with a malformation — fibroma of the tongue but there was no evidence of Recklinghausens disease malformations of the branchially derived — chromosome defects. In case No. 2 there was pea equino-varus beside the orbital cyst.

According to the literature it is rather unusual for orbital infections to arise from conjunctival structures (Burnard 1959; Duke Elder & MacFaul 1974) in the two present cases.

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*Short Communication*

**DIRECT OPHTHALMOSCOPY WITH SIMULTANEOUS  
COLOUR TELEVISION TRANSMISSION**

BY

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The authors have developed a direct ophthalmoscope with simultaneous television transmission. The ophthalmoscope is used clinically for student as well as postgraduate teaching and for intercollegial discussion of retinal changes which give rise to differential diagnostic difficulties.

**Keywords:** ophthalmoscopy — television ophthalmoscopy

Direct ophthalmoscopy with a hand held ophthalmoscope is carried out by one person at a time and co-viewing is not possible. Therefore this method is difficult to teach because the instructor cannot be completely sure that the student has observed the changes which have been pointed out to him. Television transmission has the advantage of a simultaneous reproduction of the image and for many years it has been endeavoured to obtain this advantage. Up till now the television cameras have been mounted on slit lamps and fundus cameras (a few references are obtainable from the authors) but none of these methods is based on the reproduction of the daily clinical situation in which the retina is viewed with a hand held ophthalmoscope.

On the basis of constant improvements in television technique through the past

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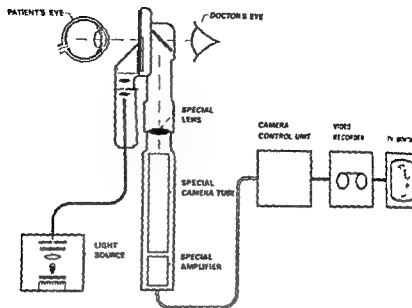


Fig 1

Schematic drawing of the television ophthalmoscope

years and in the attempt to create a clinical situation with a view to teaching intercollegial discussion we have developed a direct hand held ophthalmoscope with simultaneous colour transmission to a television screen and video recorder.

**Technical data** The apparatus consists of a light source, a light transmitter and a television ophthalmoscope, a camera control unit, a colour video recorder and a colour television monitor (Fig 1).

**Light source and light transmitter** Ordinary commercially available light sources are used. The light transmitter is a fibre light ophthalmoscope.

**The television ophthalmoscope** The light from the light transmitter is directed to the patient's eye. The light is reflected in the usual way through a lens and prism system to be focused on the retina. As in conventional direct ophthalmoscopy, the viewer sees the retina through a variable system of lenses which correct for the patient's and the examiner's refraction. The light reflected from the retina is divided by a beam splitter, partly to the examiner and partly to the television camera. In front of the television camera a lens system is inserted permitting correction corresponding to the examiner's spherical refraction anomaly.

the specifications for the television camera are

<i>Camera</i> Single tube colour camera 1 inch colour mesh	<i>Video output</i> 1 volt sync negative PAL, in compliance with CCIR 75 ohms unbalanced
<i>Camera</i> 5 × 5 × 20 cm in size	<i>Signal to noise ratio</i> Better than 40 dB
<i>Exposure</i> 25 exposures/sec	<i>Lens</i> 25 and 50 mm C mount
<i>Interface</i>	<i>Power requirement</i> 230 V ± 10% 50 Hz
<i>Horizontal frequency</i> 15 625 kHz	<i>Power consumption</i> 50 W
<i>Vertical frequency</i> 50 Hz	<i>Operating temperature</i> 0–50°C
<i>Resolution</i> Less than 1°	<i>Colour temperature compensation</i> 3200 K 4200 K 6000°K
<i>Vertical distortion</i> Less than 2%	<i>Light distribution</i> 15% to the examiner 85% to the television camera
<i>Light capability</i> 250 lines	
<i>Illuminance</i> 10 lux at diaphragm 2.5	

*Video control unit colour television monitor and videorecorder* The video signal is transmitted in the usual way to the three above mentioned units and this affords an image on the television screen simultaneously with the ophthalmoscopy. At the same time the image is recorded on a video tape for later replay.

The television ophthalmoscope is used in our Eye Department for simultaneous viewing of retinal changes when such changes are to be demonstrated for teaching purposes or when it is attempted to solve a differential diagnostic problem through intercollegial discussion. The use of videorecorders which can display films increases the usefulness of the exposures.

In particular the television ophthalmoscope is well suited for exposures of children bedridden patients or handicapped persons in whom it is difficult to photograph the fundus.

## DISCUSSION

The image quality in the television transmission can be up to that of photographs. It television images cannot be reproduced as paper prints or used for making mounting blocks of the same quality as photographs of the fundus.

Today television transmission is of such high quality with respect to resolution, stability and colour recording that by virtue of its simultaneous reproduction it is already being used instead of film for the demonstration of operations.

Due to its small size and increased sensitivity the camera and ophthalmoscope developed by us introduce for the first time the televising of direct ophthalmoscopy.

moscopy. The quality is up to that which we have seen on television through a fundus camera, but our apparatus reproduces the same effect in daily clinical practice in which the light moves over the central and peripheral parts of the retina.

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# EIGHT CASES OF CONGENITAL ACHROMATOPSIA WITH AMBLYOPIA IN TWO PEDIGREES FROM NORTHERN SWEDEN

BY

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Two families from northern Sweden with a total of 9 patients with typical symptoms of congenital achromatopsia with amblyopia were studied. In one of the families 4 affected children (3 brothers and 1 sister) also showed pallor of the optic discs and marked astigmatism. The transmission of the disease was consistent with an autosomal recessive inheritance in both families. The study confirmed that complete and incomplete achromatopsia might be different expressions of the same gene. Six out of 13 near relatives of the achromatic patients showed minor colour vision defects suggesting a tendency towards heterozygotic manifestation of the gene.

*Key words:* achromatopsia - colour vision - autosomal recessive inheritance - heterozygotic manifestation of recessive genes

Several types of so-called cone dysfunction syndromes are known among them congenital achromatopsia with amblyopia (Deutman 1971). Patients with typical congenital achromatopsia show a complete lack of colour vision and a visual acuity of about 0.1 bilaterally. They also show nystagmus - usually in childhood - and pronounced photophobia. Colour matches can be over the entire range of the Nagel anomaloscope and brightness matches only over the end of the instrument (Fig. 1). When tested with colour vision tests the achromatic patients miss most plates in the pseudo-isochromatic series and make over the entire F M 100-Hue test with no definite axis. Ocular lesions associated with achromatopsia have been recorded e.g. retinal changes, pallor of the optic discs and astigmatism. Waardenburg (1963) and others have considered pallor of the optic discs to be a familial symptom.

January 92 1979



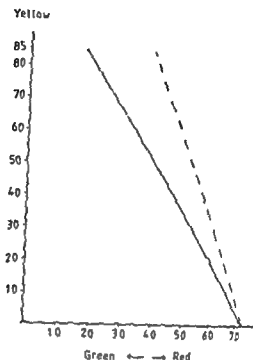


Fig. 1

Positions on Nagel anomaloscope where brightness matches can be made by achromats (---) and incomplete achromats (—) (Knoll).

although the significance of the pallor is unknown. According to Knoll genuine atrophy of the optic nerve seems unlikely.

The hereditary character of the disease is obvious. Holm and Leber presented a pedigree with 19 cases of congenital achromatopsia in a family. In Sweden Goshlin (1911) presented two pedigrees with a total of seven cases. He also discussed the heredity from the point of view of earlier reports on the disease. Congenital achromatopsia is an unusual disorder. According to Collin its prevalence is less than one in 300000. Cases have been presented in American and Japanese literature since the 1850's; these are reviewed by Francon (1961) and Wardenburg (1961).

In most cases achromatopsia seems to be complete but incomplete forms occur. In the incomplete forms the findings are not so pronounced as in the complete forms and brightness matches in the Nagel anomaloscope are also possible in the green section (Fig. 1). According to Franceschetti et al. (1957) complete and incomplete achromatopsia may be different expressions of the same gene. They found the complete type in an elder and the incomplete type in a younger brother of the Danish family presented earlier by Holm & Leber (1957).

naka (1957) suggested an X-chromosome linked transmission of the disease. According to Deutman (1971) incomplete achromatopsia can be X linked recessive, possibly also autosomal recessive, while complete achromatopsia always has an autosomal recessive mode of inheritance.

Alford (1957a, 1959) found deviation of red-green colour vision and abnormal brightness ranges in 3 parents of 2 patients with congenital achromatopsia when tested with the anomaloscope, indicating a tendency towards manifestation of the gene in a single dose.

Deutman (1971) stressed the importance of more information on families with dysfunctions in order to establish the mode of inheritance and to delineate this condition more sharply from other similar conditions.

The aim of the present investigation was

to describe the clinical manifestation of the disease in two hitherto unpublished families and

to study the mode of inheritance including manifestation of the gene in heterozygotes.

## Materials and Methods

Four out of 6 brothers and sisters in one family (Fig. 2) and 3 out of 4 in another family (Fig. 3) were affected by the disease. Both families lived in the same parish (Körola) in the county of Västerbotten in Northern Sweden. Another patient (III-1, Fig. 9), first cousin of the patients in the first family, was found in the course of the study. She and her family lived in Southern Sweden.

The 8 patients were examined ophthalmologically as follows:

Visual acuity and fundus examination

Colour vision testing with

- a) Bk and BII pseudoisochromatic plates
- b) Ishihara plates (38 plates edition 1972)
- c) American Optical Hardy Rand Rittler (HRR) plates
- d) Farnsworth Munsell (F M) 100 Hue test
- e) Anomaloscope (brightness matches)

The 6 parents and all the clinically normal brothers and sisters of the achromatopsia patients were examined with the aim of detecting any possible heterozygotic manifestation of the gene. One aunt of the second family and her son were also included. This examination was performed as described above with the following conditions:

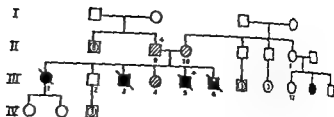


Fig 2  
Pedigree of Family I

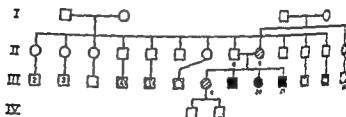
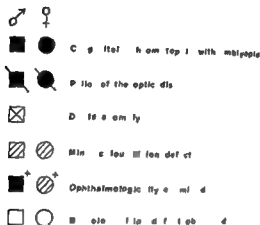


Fig 3  
Pedigree of Family II

2 e) Anomioscope. Red green deviation and matching range. The test was made with a Nagel anomioscope calibrated according to Pickford (1973). The anomioscope was calibrated by examination of 30 persons (15 men and 15 women) with normal colour vision and without known near relative vision defects. The limits at which the red green variable had to be greener than the yellow standard was scale number 44 (only 9 persons) and 41 (7 persons) respectively. Most settings lay between scale number 44 and 41.

The brightness was always held equal by appropriate changes of the intensity of the yellow. Matching range varied between two and four scale units of the achromats (III 1 Fig 2) had 2 daughters who were clinically normal but were however too young to cooperate in a detailed colour vision examination.

## Results

affected patients in the same sibship of family I (Fig 2) had a visual acuity of moderate astigmatism photophobia nystagmus and pallor of the optic discs. First cousin in family I (III 14) had a visual acuity of 0.2 moderate astigmatism photophobia nystagmus but no pallor of the optic discs. In family II



Fig 4

Brightness matches in Nagel anomaloscope made by the five achromats in Family I (Fig 2)

- ◇ = III 1 (aged 31)
- = III 3 (aged 29)
- = III 5 (aged 20)
- × = III 6 (aged 15)
- = III 14 (aged 13)

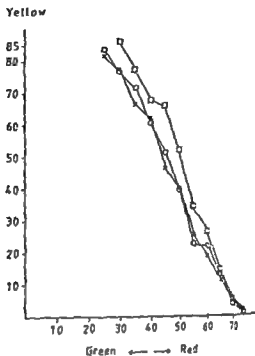


Fig. 5

Brightness matches in Nagel anomaloscope made by the three achromats in family I.

- O = III 19 (aged 20)  
 □ = III 20 (aged 11)  
 x = III 21 (aged 11)

(Fig. 3) the 3 affected patients had the same typical symptoms of achromatopsia: visual acuity 0.1, photophobia, nystagmus, marked astigmatism, accommodation reflexes but no pallor of the optic discs. When tested with pseudochromatic plates all the 8 patients missed most plates in all series. All of them read plate 14 of the Ishihara test correctly. (The plates 14 and 15 are also the first to be missed by dark-adapted persons with normal colour vision when the luminance is increased). Large errors were made over the entire F.M. 100-Hue test, no definite axis. Anomaloscopically all could do brightness matches in the green section (Fig. 4 and 5). There was a slight difference between the three achromats in family I (Fig. 4).

The clinically normal relatives who were examined all had normal visual acuity and normal fundi. Some had a slight myopia and hyperopia but no significant astigmatism. They all read Bk and BII Ishihara and Plate 14 of the F.M. 100-Hue test. When tested with F.M. 100-Hue test the 2 parents in family I (II 1 and II 2) and the aunt in family II (II 13) made significant errors with the green section.

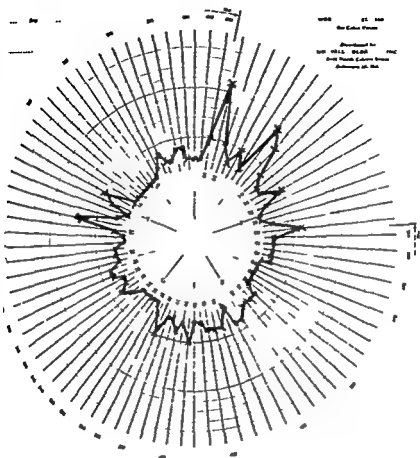


Fig 6

The Farnsworth Munsell 100-Hue error pattern of II 9 in Family I Error score 114

(Fig 6 7 and 8 Tables I and II) They had error scores which exceeded the 95 percent limit in the age group 50-59 given by Vernest (1963) and Krill & Inenderman (1964) The other relatives had normal error scores

When tested with the anomaloscope (red green) the 2 parents (II 9 and 10) and sister (III 4) in family I (Fig 2) and the mother (II 9) the sister (III 18) and aunt (II 13) in family II (Fig 3) had minor defects (Tables I and II)

The transmission of achromatopsia in the pedigrees is consistent with an autosomal recessive inheritance (see Fig 2 and 3)

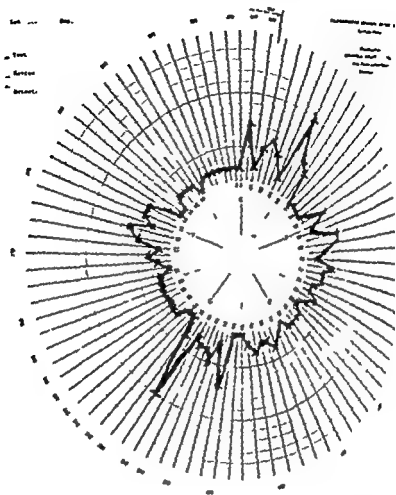


Fig. 2

The Farnsworth Munsell 100-Blue error pattern of III 14 (Fig. 2) (see text).

### Discussion

This study confirms that complete and incomplete achromatopsia are different expressions of the same autosomal recessive gene. The main finding is achromatopsia in family I, especially the first cousin (III 14) (Fig. 2) who has a more incomplete form. She had better visual acuity and could recognize colour when seen on larger objects. We also think that this study confirms that the achromatopsia gene has a tendency towards heterozygote manifestation and near relatives had minor colour vision defects. Pickford (1971) found that brown

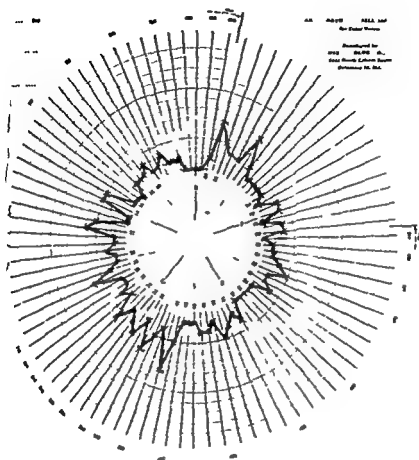


Fig 9

Fig. 5  
The Farnsworth Munsell 100-Hue error pattern of II 13 in Family II. Error score 136

for red-green blindness had minor defects which accord in character with of major defect of their affected relatives. In our study we found that a cousin (III 28 Fig 3) had a deuteranomaly. His mother (II 13) and aunt ) however showed minor defects with red deviation. It is more likely that this festation of the gene for achromatopsia than of that for deuteranomaly. F M 100-Hue test may sometimes be useful in detecting heterozygotes with a gene. Tests on larger numbers of near relatives of patients with matopsia may confirm this.



Table 1  
Results of test on near relatives of achromats (Fig. 5)

Case	Anomaloscope test (red-green)	Result
II 9 (aged 57)	Green deviation (scale number 36) Matching range abnormal (36-44) 9 scale units	Abnormal (Fig. 1)
II 10 (aged 53)	Green deviation (scale number 35) Matching range abnormal (35-43) 7 scale units	Abnormal (Fig. 1)
II 13 (aged 57)	Deviation not abnormal Matching range normal (40-43)	Normal
II 14 (aged 41)	Deviation not abnormal Matching range normal (39-40)	Normal
III 2 (aged 51)	Deviation not abnormal Matching range normal (41-42)	Normal
III 4 (aged 25)	Green deviation (scale number 36) Matching range abnormal (36-40) 5 scale units	Abnormal (Fig. 2) [Fig. 2b] normal
III 13 (aged 16)	Deviation not abnormal Matching range normal (39-42)	Normal
III 15 (aged 11)	Deviation not abnormal Matching range normal (41-42)	Normal

A genealogical study of the two families (Nordström & Pollard) has shown that the gene causing the disease has existed for at least 300 years in the subpopulation in northern Sweden.

Table II  
Results of test on near relatives of achromats (Family II)

Case	Anomioscope test (red green)	FM test
II 8 (aged 52)	Deviation not abnormal Matching range normal (38-41)	Normal
II 9 (aged 51)	Red deviation (scale number 48) Matching range abnormal (39-49) 10 scaleunits	Normal
II 13 (aged 54)	Red deviation (scale number 47) Matching range abnormal (38-47) 9 scaleunits	Abnormal (Fig 8)
III 18 (aged 93)	Red deviation (scale number 46) Matching range abnormal (38-46) 8 scaleunits	Normal
III 99 (aged 94)	Deuteranomaly	

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## RACEMOSE HAEMANGIOMA OF THE RETINA

Report of three cases with long term follow up

BY

PETER BERNTH PETERSEN

*Racemose haemangomas of the retina are rare developmental anomalies. The basic lesion is an abnormal arterio-venous communication.*

*Three new cases with long term follow up are presented. In two of the cases the first symptom was loss of vision due to retinal and vitreous haemorrhage. In the third case gradual reduction of vision was due to vascular leakage into the macular area. The recent literature is reviewed and the association to midbrain haemangiomas discussed. The ophthalmological differential diagnosis, the prognosis and the present status in treatment is presented.*

*Key words:* racemose haemangomas of retina - arterio-venous aneurysm of retina - developmental anomalies - intracranial arterio-venous aneurysm - retinal vascular decompensation

Racemose haemangomas of the retina are rare developmental anomalies. Since Knapp in 1874 described the condition, less than 80 cases have been reported, a number of these from Denmark (Ehlers 1924, Frandsen 1950, Bech & Jensen 1958, Petersen 1961).

The ophthalmoscopic picture varies from a single well-defined anastomosis to a tumour-like mass of arteries and veins. Probably for this reason the condition has been described under a number of terms including racemose angioma, arterio-venous angioma, arterio-venous aneurysm and cirroid aneurysm. The condition is not a true neoplasm, as it has no potential for unlimited growth, production of metastases or infiltration, nor does any characteristic stem cell exist (Reese 1976). The term aneurysm is in fact also misleading as aneurysmal

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Racemose haemangiomata of the retina are rare developmental anomalies. The basic lesion is an abnormal arterio-venous communication.

Three new cases with long term follow up are presented. In two of the cases the first symptom was loss of vision due to retinal and vitreous haemorrhage. In the third case gradual reduction of vision was due to vascular leakage into the macular area. The recent literature is reviewed and the association to midbrain haemangiomas discussed. The ophthalmological differential diagnosis, the prognosis and the present status in treatment is presented.

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The ophthalmoscopic picture varies from a single well-defined anastomosis to a fleshy tumour-like mass of arteries and veins. Probably for this reason the condition has been described under a number of terms including racemose angioma, arterio-venous angioma, arterio-venous aneurysm and cirroid aneurysm. The condition is not a true neoplasm, as it has no potential for unlimited growth, production of metastases or infiltration, nor does any characteristic stem cell exist (Reese 1976). The term aneurysm is in fact also misleading, as aneurysmal

dilatations are not the primary lesion although they occur secondary to large arterio-venous communications

The essential defect is one or more abnormal communications between retinal arterial and venous circulation probably due to an error in the anterior plexus of primordial cerebral blood vessels (Rabek & Archer et al 1973 Reese 1976 Speiser 1978)

In two recent papers the term arterio-venous communications have been suggested for this condition (Baurmann et al 1980 Uthman

A report of three new cases of racemose haemangioma is given below

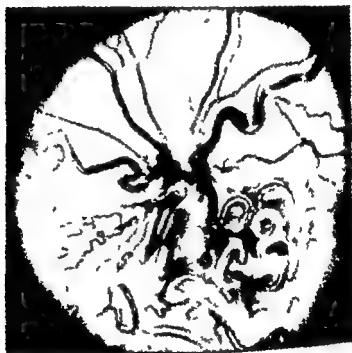


Fig 1

Central part of fundus of the left eye in case No 1  
A group 3 racemose haemangioma

Fig 2

Early midphase fluorescein angiogram of disc area and part of the lower  
lower temporal quadrant of left eye (Case No 1) A racemose haemangioma

Fig 3

Central part of fundus of the left eye in case No 2 with a large racemose haemangioma  
White sheathings of some of the vessels



*Racemose Hemangioma of the Retina*



## Case Reports

### Case 1 (52 08 14)

A woman aged 19 years was admitted to the Department of Ophthalmology under the diagnosis of phacomatosis previously in regard to both eyes. In the past few eye diseases the patient one month before the admission sudden vision loss occurred in the right eye. Since then the reduced vision in the right eye has remained unchanged.

Objectively normal external eye conditions were found. Left pupil dilated and the right both reacted normally to light. Visual acuity was 10 in the right eye and 20 in the left eye. Tension 14/12 mmHg. Normal visual fields. Normal glaucoma test in the right eye. In the left eye ophthalmoscopy showed dilated and a mass tumour on the fundus and in the lower quadrants just below the disc a tumour. The tumour was highly tortuous vessels forming loops with a 2-3 diameter per centimeter. The arteries and veins could not be differentiated. No pulsation was seen in the macula. No DD haemorrhage and in the lower temporal quadrant two distinct and scattered small haemorrhages. Fluorescein angiography showed rapid filling of the tumour with no dye leakage (Fig. 2).

Fig. 4



Figs. 4, 5, 16

Early, mid and late phase fluorescein angiogram of the area with part of the tumour. Rapid filling of all vessels in the haemangioma and in Fig. 16 the vessels in the border of the tumour at 1 in the macula area.



Neurological examination revealed hyperactive reflexes (H) was (irregular 5-7 Hertz activity) temporally on both sides. Radiography of the foramen and left orbit was normal. Left carotid angiography showed no vascular malformations in the brain. The patient was seen in this department in 1973 and at left eye January 1979 vision and ophthalmoscopy were unchanged. At left eye angiography had been performed in 1972 and found normal. CT scan performed at the first examination. No treatment was offered.

## Case 2 (26.06.20)

A woman aged 48 years previously of good health and suffer a vision loss in the left eye before admission in November 1974 she experienced a sudden loss of vision in the left eye.

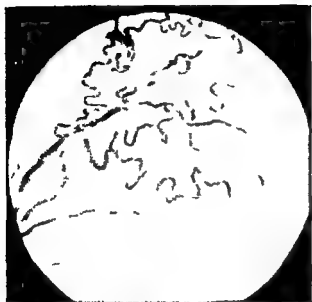
Objective examination showed moderate telephthalosia and exophthalmos. Left eye fissure was a little bigger than right. No exophthalmos was present. Visual acuity in left eye 0.33 right eye 1.0. Ophthalmoscopy showed a normal fundus and hypoplastic disc and just above this a tumour extending into the vitreous. Vessels forming prominent loops (Fig. 3). Arteries and veins could not be seen clearly and no pulsation was seen. From the disc three large dilated and sheathed vessels passed through the vessel conglomerate into the vitreous. Scattered in the fundus some small haemorrhages were seen and in the lower part there was a considerable vitreous haemorrhage. Visual field examination showed corresponding to the haemorrhage. Normal ophthalmoscopy fundus. Fluorescein angiography showed rapid filling of all vessels in the retina and some dye leakage in the retina at the temporal border of the tumour (Figs. 4, 5, 6). Radiography of the skull and chest and general examination was normal.

The following years this patient had 7 or 8 periods with slight pain and loss of vision in the left eye. At these periods the patient's ophthalmoscopy showed exophthalmos and sometimes slight haemorrhage in the vitreous, and treated with prednisolone and steroids. According to the ophthalmologist the haemorrhage was not a vitreous haemorrhage in the vitreous appeared in September 1978 with severe loss of vision.

The patient was again admitted to the department in December 1978. Vision in the left eye was 1.0 and in the left eye vision was reduced to finger movement. The fundus was a little larger than the right. No exophthalmos was present. The fundus in both eyes and the pupils were normal. At slit lamp examination a small vitreous haemorrhage was seen in the left eye. The fundus could not be seen at the bottom. Normal retina in the right eye. CT scanning of the skull and the lower part of the spine was abnormal. No treatment was offered.

Fig. 7  
Upper temporal part of fundus of the left eye in case 2  
A group 2 tumour with haemorrhage.

Fig. 8  
Mid phase fluorescein angiogram of macula area of the left eye in case 2  
dye leakage and abnormal vessels.



*Fig 7*



*Fig 9*

## Case 3 (36 01 02)

A woman aged 35 years was admitted to the Department of Ophthalmology in 1971. Previously she had been healthy except for a subconjunctival haemorrhage in the left eye at the age of 19 years. During the year prior to admission there was a progressive decreasing vision in the left eye. Three months earlier there was a sudden decrease in the vision of the left eye.

Objectively: normal external eye conditions and pupils were found. In the right eye and 0.3 in the left eye. Normal ophthalmoscopy in the right eye showed dilated and tortuous vessels were seen in the entire fundus especially in the upper temporal quadrant where the vessels formed a small conglomerate two DD below the macula. The vessels and no pulsation of the vessels was seen. No exudates and no haemorrhages. The vessels in the upper temporal quadrant had a whitish sheath. In the left eye examination showed a central scotoma and several scattered small haemorrhages. Angiography showed rapid filling of the conglomerate of vessels in the upper temporal quadrant and dye leakage in the macula area (Fig. 8). Neurological examination and EEG radiography of the skull was normal. Radiographs of the left orbit showed thinning in the back wall of orbita. Therefore a left sided craniotomy was performed but it was normal without any signs of intracranial haemorrhage.

At the follow up examination in January 1979 the patient indicated a further decrease in vision of the left eye. There were no other ophthalmological or neurological changes. In the right eye 0.4 in the left eye 1.0. Normal retina in the right eye unchanged haemorrhages in the left eye. Carotid angiography was performed in 1971 and found to be normal. CT scanning of the brain was not performed at this examination. No further changes.

## Discussion

The ophthalmoscopic findings in the two cases with a localized racemose composed of large tortuous vessels is the one most often reported. The deterioration of vision which appeared in two of the patients is a late manifestation of the racemose haemangioma. On basis of the ophthalmoscopic and angiographic pictures and of growth pattern different classifications have been suggested (Rundles & Falls 1951, Cameron 1958, Reese 1971, Riva 1973). Riva (1973) studying structural abnormalities and alterations in retinal hemodynamics by suction-cup ophthalmodynamometry combined with fluorescein angiography classified three groups of arterio-venous communications. Group II is characterized by the interposition of small caliber arterio-venous communications between the major vessels. It remains stationary and does not affect retinal hemodynamics or the vision.

The second group includes arterio-venous anastomoses of large caliber without aneurysmal dilations. The venous side of the communications is characterized by hyperdynamic flow and high intraluminal pressure. This is associated with exudates, oedema, venous thrombosis and haemorrhages. In the third group arterio-venous anastomoses are of very large caliber and aneurysmal dilations.

involved vessels which become convoluted and intertwined. Some of them develop fibrous sheathings. The venous side of the system suffers from high intraluminal pressure causing retinal haemorrhages and oedema with severe loss of vision. The patients in this group are claimed to have a higher incidence of intracranial arterio-venous aneurysms than the normal population. Using this classification case No. 1 and No. 2 should be placed in the third group and case No. 3 in the second group.

Besides the complications mentioned above other symptoms have been described: exophthalmus, strabismus, anisocoria (as in case No. 1), ptosis and myopia (Baurmann et al. 1968). The association with intracranial arterio-venous aneurysm is a well known and controversial subject. The co-existence was particularly emphasized by Wyburn-Mason (1943) who found a very high incidence (50%) of signs of intracranial aneurysms in the midbrain in patients with retinal racemose haemangioma. Bech & Jensen (1961) pointed out in a review of the literature that this figure was much too high and considering the relationship between the aneurysms of the two regions from the reverse angle showed that only 10% of the patients with verified cerebral aneurysm had racemose haemangiomas of the retina. This has been confirmed in later studies (Cagianut 1962, Linger & Kuch 1966).

It is now recommended that patients with racemose haemangioma in the retina should be examined neurologically and if possible a CT-scanning of the brain and spine should be done. Only if these examinations raise suspicion of intracranial aneurysms should further neuroradiological examinations be performed. None of the cases in this paper showed signs of intracranial haemangiomas at cerebral angiography or CT scanning.

Among the conditions to be considered in the differential diagnosis most authors mention von Hippel's disease (Angiomatosis retinalis) and von Hippel-Lindau's disease (Angiomatosis cerebro-retinalis). The major features in these syndromes are multiple tumours in the retina with large afferent and efferent vessels and no communications between the vessels. In the Sturge-Weber-Dimitri disease the clinical signs may resemble those of the racemose haemangioma but the choroidal angiomas, the naevus flammeus and the absence of arterio-venous anastomosis may be helpful in the differentiation. In some diseases vascular communications may develop as a result of prolonged vascular stasis e.g. arterial embolism, chronic glaucoma, central venous thrombosis, retinal haemorrhage or after chorioretinitis. These conditions are rarely confused with true haemangiomas.

The course of this disease varies a great deal from case to case. The first manifestations of the racemose haemangioma usually occur in the second and third decades of life. If the haemangiomas are rather small (group I or II in the author's classification) they probably do not show any or only slight signs of

progression as in case No 1 and No 3 although signs of leakage may occur from time to time. In a few cases spontaneous leakage has been reported (Gregersen 1966). The prognosis of the large haemangioma is generally poor as in case No 2.

Owing to the good prognosis in most cases treatment is not indicated in selected cases. Different surgical methods have been tried (Wiburn 1976). In some recent papers photocoagulation has been recommended for patients with leakage exudates or haemorrhage and consequent reduced vision where no signs of spontaneous resolution have been seen (Cogan et al 1968, Archer et al 1973). This treatment has however not been tried in a few patients in order to close the abnormal communication of retinal vessels to afferent artery. Generally photocoagulation of such large vessels is very dangerous with risk of severe haemorrhages. Considering the indications and the hazards of this therapy photocoagulation treatment is not to be indicated in case No 1 and No 3. In case No 2 photocoagulation is a practical proposition at the present time because of the increased risk of blurring the insight to the retina.

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## AGE VARIATIONS IN NORMAL HUMAN CONTRAST SENSITIVITY

BY

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The visual contrast sensitivity (the reciprocal of contrast threshold) was studied as a function of age. Psychophysical measurements of binocular and monocular contrast thresholds were made for 33 normal observers at spatial frequencies within the range 0.5 to 40 cycles/degree. The observers were divided into three different age groups: young, middle aged, and old subjects with the age ranges 6–10 years, 20–40 years, and 60–70 years respectively. All observers had healthy eyes, normal vision, and Snellen visual acuity of 1.0 or better in both eyes.

In all groups, contrast sensitivity for binocular and monocular viewing peaked at a spatial frequency around 3–5 cycles/degree and showed the typical attenuation at low and high spatial frequencies. The binocular contrast sensitivity was higher than the monocular.

There was no significant difference between young and middle aged subjects with regard to contrast sensitivity. Subjects aged 60 years or more showed significantly lower contrast sensitivity than younger subjects for most spatial frequencies above 4 cycles/degree. We may thus conclude that both the binocular and monocular contrast sensitivity seemed independent of age within the range of 6 to 40 years. For higher ages studied (above 60 years) there was a loss of sensitivity in the middle and high frequency regions.

*Key words:* human contrast sensitivity — sinusoidal gratings — age dependence

Measurement of contrast sensitivity to sinusoidally modulated gratings (Campbell 1966; Campbell 1973) has become clinically important as a method of detecting visual dysfunction (Arden 1978). This psychophysical method has been

employed in studies on the visual effects of optical deterioration in cataract (Hess & Garner 1977) and cataract (Hess & Woot 1978), in glaucoma (Jacobson 1978), visual dysfunction in macular disease (Systrand & Frisén 1978), optic neuritis (Regan et al. 1977, Frisén & Systrand 1978), in meridional amblyopia (Mitchell et al. 1973, Frisén & Systrand 1978), in anisometropic or strabismic amblyopia (Spekreijse et al. 1977, Hess & Smith 1977, Levi & Harwerth 1977, Systrand 1978), in visual disturbances due to cortical lesions (Bodis Wollner 1977, Levi & Harwerth 1977, Diamond 1976).

Thus many pathological states have been examined but the results have not been related to only a few normal subjects or subjects not different from normal subjects to age in each investigation. In a study by Arundale (1978) on the age dependence of contrast sensitivity only a few young and old subjects were examined. In a study by Jacobson (1978) found a lower sensitivity in subjects over 40 years of age than in younger subjects but the range of spatial frequencies tested was rather narrow.

As a basis for clinical studies monocular and binocular contrast sensitivity was measured in young, middle aged and old subjects with normal vision. The contrast thresholds were determined for a wide range of spatial frequencies using different techniques but employing a higher luminance level than in the previous studies.

## Materials and Methods

### Methods

The visual stimuli presented were vertical gratings displayed on a cathode ray oscilloscope (Tektronix 5410 with 131 phosphor). By means of a video amplifier the signal from a function generator (Tektronix FG 501) an even harmonic signal was produced. The beam was intensity modulated with another function generator (Exact 129) producing a sinusoidal luminance profile on the screen. The contrast could be varied continuously by means of an attenuator. The luminance of the oscilloscope was sensed by a peak detector and the contrast value was displayed. Contrast was defined as  $L_{max} - L_{min} / L_{max} + L_{min}$ . The maximum luminance used was 0.19. The display subtended  $4.5 \times 5.0$  degrees of visual angle at a viewing distance of 143 cm which was kept constant by means of a chinrest. The mean luminance of the screen was 1.0 cd/m<sup>2</sup>. The screen was illuminated by an ordinary desk lamp containing a 60 W incandescent bulb with a surround luminance of about 20 cd/m<sup>2</sup>. The subject was adapted to the luminance for at least 10 min before the experiment.

jects

thirteen normal observers with ages in the range 6 to 70 years participated in the investigation. They were divided into three age groups: one of 10 subjects between 6 and 10-years-old (8 girls and 2 boys), one of 12 subjects between 20 and 40-years-old (10 women and 2 men), and one of 11 subjects of 60 years or older (8 women and 3 men). A smaller group of 5 subjects (3 men and 2 women) between 40 and 60-years-old was also examined. No one had any previous experience in threshold experiments. Eyes and vision were normal in all subjects as revealed by ophthalmological examination including careful refraction and binocular assessment. All subjects had Snellen visual acuity of 1.0 or better in both eyes and could detect stereoscopic disparities down to 40 sec of arc. Subjects above 60 years of age were given +0.5 sph in addition to their static refraction in order to compensate for the viewing distance. Normal pupils were used.

procedure

The contrast thresholds were determined binocularly and for each eye alone at 19 spatial frequencies ranging between 0.5 and 40 cycles/degree. The threshold was determined by raising the contrast from a subthreshold value at a selected spatial frequency until a grating pattern was just detected on the screen. The contrast was varied continuously by the experimenter by means of a potentiometer. We have used this most commonly employed method and not the reverse procedure, i.e. lowering the contrast from a supraliminal level which might induce disturbing masking/adaptation phenomena (Blakemore & Campbell 1969). The training of the subjects before the experiment included presentations of gratings well above threshold contrast in the low, middle and high frequency regions and a few threshold determinations. Intermittently during the testing the subjects were asked to give an estimate on the grating density of the pattern which could just be detected. This was used as a control of their reports on pattern recognition. The spatial frequencies were presented in a random order. The initial sessions were performed in the middle aged subjects who in binocular viewing made five judgements per frequency. The values were very consistent for each subject and for the rest of the binocular testings three judgements per frequency were considered sufficient. During the monocular viewing all subjects made two judgements for each frequency. For the binocular viewing the session lasted about one hour for each subject in the middle aged group and about half an hour in the two other groups. For the monocular viewing condition the session lasted approximately 20 min for each eye. During the monocular testing the other eye was covered with a black occluder. The binocular and monocular testing was mostly carried out on separate occasions. Four different experimenters have been involved in the testings, each examining subjects of at least two age groups.

## Results

For each subject the contrast sensitivity (the reciprocal of the mean of contrast values) was plotted against spatial frequency for best and distance viewing. Examples of individual curves typical of each age group are given. The curves characterising the contrast sensitivities were quite similar.

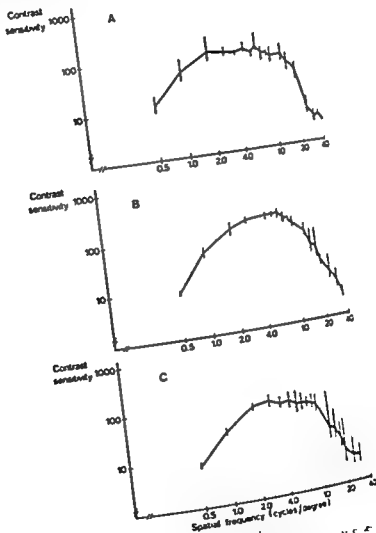


Fig. 1  
Monocular contrast sensitivity of an eight-year-old boy (A), a twenty-year-old woman (B) and a sixty-one-year-old woman (C) plotted against spatial frequency. Data points are the reciprocal of the arithmetic mean of four threshold values (two for the left and two for the right eye). Bars represent  $\pm 1$  standard deviation.

# Contrast Sensitivity and Age

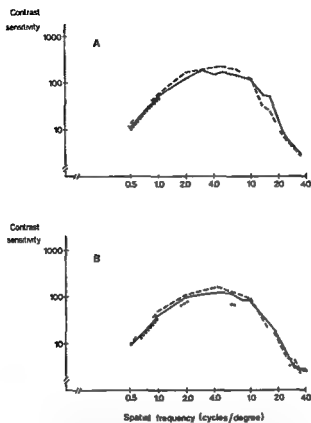


Fig 2

Binocular contrast sensitivity for three age groups (— 6-10 years      - - - 10-20 years      . . . 20-40 years) plotted against spatial frequency in log log coordinates. Data points are the reciprocal of the average binocular contrast thresholds for all individuals in each group (see Table I)

The same as in A but for monocular contrast sensitivity

the children. The variations between measurements at each spatial frequency was about the same at all ages as seen in Fig. 1. The curves most often peaked at spatial frequencies of 3-5 cycles/degree. They were typically attenuated at low and high frequencies. They were very similar for the right and the left eye. The monocular contrast sensitivity given in Fig. 1 is therefore an average value of the results from both eyes. The binocular contrast sensitivity was generally higher than the monocular one.

The contrast sensitivity (binocular and monocular) for each age group is plotted against spatial frequency in log log coordinates in Fig. 2. For all groups the curves had a peak sensitivity at a spatial frequency around 3-5 cycles/degree. Contrast

Table I

Binocular threshold contrast (means and SDs determined for 12 subjects per age group) from 0.5 to 40 cycles/degree. The contrast sensitivity (reciprocal of the threshold contrast) is also given. The values are given for each age group: No. 1 = 10-19 years, No. 2 = 20-40 years, No. 3 = 60-70 years. The F ratios from the analysis of variance for each spatial frequency.

Spatial frequency cycles/degree	Age group	Threshold contrast		Contrast sensitivity	Number of subjects	
		M	SD			
0.5	1	0.1003	0.0221	9.97	10	
	2	0.0950	0.0727	10.53	10	$P > 1$
	3	0.1075	0.0318	9.30	11	
1.0	1	0.0194	0.0045	51.41	10	
	2	0.0188	0.0095	53.31	10	$P < 1$
	3	0.0227	0.0087	44.15	11	
2.0	1	0.0041	0.0039	193.61	10	
	2	0.0065	0.0018	151.44	10	$P < 1$
	3	0.0100	0.0035	100.00	11	
3.0	1	0.0056	0.0017	177.31	10	$P < 1$
	2	0.0052	0.0011	191.57	10	$P > 1$
	3	0.0067	0.0012	149.40	11	
4.0	1	0.0067	0.0027	150.15	10	$P < 1$
	2	0.0047	0.0013	212.77	10	$P < 1$
	3	0.0076	0.0024	131.54	11	
5.0	1	0.0058	0.0014	172.41	10	
	2	0.0045	0.0010	222.00	10	$P < 1$
	3	0.0080	0.0020	125.00	11	
6.0	1	0.0069	0.0020	145.00	10	
	2	0.0045	0.0012	222.4	10	$P < 1$
	3	0.0091	0.0025	109.45	11	
7.0	1	0.0064	0.0016	155.4	10	
	2	0.0057	0.0011	175.55	10	$P < 1$
	3	0.0102	0.0031	97.22	11	
8.0	1	0.0072	0.0022	137.08	10	
	2	0.0058	0.0014	173.16	10	$P < 1$
	3	0.0117	0.0029	85.91	11	
9.0	1	0.0075	0.0021	133.63	10	
	2	0.0067	0.0015	149.0	10	$P < 1$
	3	0.0115	0.0015	86.94	11	

# Contrast Sensitivity and Age

Table I (cont.)

Spatial frequency /degree	Age group	Threshold contrast		Contrast sensitivity	Number of subjects	F
		M	SD			
0.0	1	0.0082	0.0027	122.10	10	11.32
	2	0.0085	0.0026	117.07	12	$P < 0.01$
	3	0.0165	0.0071	60.71	11	
1.0	1	0.0174	0.0083	55.93	10	3.38
	2	0.0291	0.0277	34.33	12	$P > 0.01$
	3	0.0478	0.0352	20.91	11	
16.0	1	0.0190	0.0068	52.77	10	12.41
	2	0.0373	0.0262	26.82	12	$P < 0.01$
	3	0.0883	0.0505	11.32	11	
18.0	1	0.0506	0.0548	19.77	10	3.63
	2	0.0592	0.0420	16.90	12	$P > 0.01$
	3	0.1248	0.1007	8.02	11	
20.0	1	0.0749	0.0571	13.34	10	7.46
	2	0.0867	0.0531	11.54	12	$P < 0.01$
	3	0.1599	0.0567	6.26	11	
25	1	0.1633	0.0541	6.19	10	2.77
	2	0.1706	0.1070	5.86	11	$P > 0.01$
	3	0.2474	0.0856	4.04	9	
30.0	1	0.2498	0.1089	4.00	10	1.21
	2	0.2360	0.0843	4.24	8	$P > 0.01$
	3	0.3363	0.0836	2.97	3	
35.0	1	0.3146	0.0693	3.18	7	1.31
	2	0.3043	0.0899	3.29	4	$P > 0.01$
	3	0.4300		2.33	1	
40.0	1	0.2117			1	

Sensitivity curves of men and women showed no differences within each age group. Contrast sensitivity and the group means of the monocular and binocular contrast thresholds are given in Tables I and II for each age group. Both the monocular and the binocular data show that the old age group has a lower contrast sensitivity i.e. higher contrast thresholds for all spatial frequencies compared to two younger age groups. As shown by an analysis of variance and *t* tests of the

Table II

Monocular threshold contrast (means and SDs determined for 15 subjects) for spatial frequencies from 0.3 to 40 cycles/degree. The contrast sensitivity (reciprocal of the threshold contrast) is also given. The values are given for each age group No. 1 = 16-20 years, No. 2 = 20-40 years, No. 3 = 60-70 years. The F ratios from the analysis of variance for each spatial frequency.

Spatial frequency cycles/degree	Age group	Threshold contrast		Contrast sensitivity	Analysis of variance	
		M	SD		F	P
0.3	1	0.1051	0.0071	9.57	12	
	2	0.0997	0.0273	10.03	12	P<
	3	0.1418	0.0371	7.02	11	
1.0	1	0.0213	0.0101	41.53	12	
	2	0.0205	0.0101	48.96	12	P<
	3	0.0372	0.0077	30.11	11	
2.0	1	0.0096	0.0017	104.53	12	P<
	2	0.0085	0.0027	117.73	12	P<
	3	0.0123	0.0027	81.5	11	
3.0	1	0.0035	0.0005	317.19	12	P<
	2	0.0072	0.0015	137.8	12	P<
	3	0.0109	0.0026	91.14	11	
4.0	1	0.0083	0.0034	121.01	12	P<
	2	0.0063	0.0013	159.36	12	P<
	3	0.0102	0.0021	74.52	11	
5.0	1	0.0050	0.0023	174.53	12	P<
	2	0.0062	0.0017	161.32	12	P<
	3	0.0120	0.0024	83.13	11	
6.0	1	0.0083	0.0027	120.19	12	P<
	2	0.0071	0.0015	141.84	12	P<
	3	0.0126	0.0032	79.14	11	
7.0	1	0.0090	0.0024	111.96	12	P<
	2	0.0074	0.0015	133.29	12	P<
	3	0.0141	0.0045	69.35	11	
8.0	1	0.0106	0.0022	94.18	12	P<
	2	0.0081	0.0017	118.02	12	P<
	3	0.0167	0.0035	60.04	11	
10	1	0.0111	0.003	89.01	12	P<
	2	0.0092	0.0025	108.2	12	P<
	3	0.0191	0.0044	52.16	11	



*Contrast Sensitivity and Age*

*Table II (cont.)*

Spatial frequency cycles/degree	Age group	Threshold contrast		Contrast sensitivity	Number of subjects	F
		M	SD			
10.0	1	0.0125	0.0038	79.75	10	13.16
	2	0.0115	0.0096	86.73	10	$P < 0.01$
	3	0.0240	0.0095	41.69	11	
14.0	1	0.0281	0.0167	35.61	10	5.85
	2	0.0373	0.0399	96.80	10	$P < 0.01$
	3	0.0833	0.0568	12.00	11	
16.0	1	0.0385	0.0281	25.98	10	8.5
	2	0.0468	0.0340	21.37	10	$P < 0.01$
	3	0.1385	0.0951	7.29	11	
18.0	1	0.0531	0.0228	18.84	10	11.57
	2	0.0615	0.0466	16.26	10	$P < 0.01$
	3	0.1728	0.0961	5.79	11	
20.0	1	0.0925	0.0322	10.81	10	7.12
	2	0.1231	0.0695	8.13	10	$P < 0.01$
	3	0.1942	0.0749	5.15	10	
25.0	1	0.1948	0.0542	5.13	10	4.10
	2	0.1962	0.1443	5.10	9	$P > 0.01$
	3	0.3103	0.1108	3.22	8	
30.0	1	0.3198	0.0906	3.13	10	4.08
	2	0.2145	0.0409	4.66	6	$P > 0.01$
	3	0.2518	0.0408	3.97	3	
35.0	1	0.3083	0.0689	3.24	6	1.08
	2	0.3425	0.0538	2.92	5	$P > 0.01$
	3	0.3998		2.50	1	
40.0	1	0.3284	0.0716	3.05	5	0.52
	2	0.3670	0.0064	2.73	2	$P > 0.01$

threshold values this difference was significant at the 0.01 level (see Table I and II ratios) for most frequencies between 5.0 and 20.0 cycles/degree. In the low spatial frequency range (below 4 cycles/degree) this difference was significant (*t* tests) between the old and middle-aged subjects for monocular viewing. The binocular values showed no significant differences between age groups in this frequency range.

In the low and the middle frequency region (0–10 cycles/degree) the sensitivities of the children tended to be lower than those of the adult group (Fig. 2). At higher spatial frequencies the trend was reversed. As shown by *t*-tests there was no significant difference between the two groups at any spatial frequency. In the highest spatial frequency range (30 and above) no significant differences were seen between any of the groups. A few old subjects were able to detect the gratings at the highest frequencies (Tables I and II).

As a control of these results we have tested five subjects (4 younger than 60 years). They showed contrast sensitivity curves with slightly lower values than 20–40 year old subjects in the higher spatial frequency range (30–60 cycles/degree).

In order to compare monocular and binocular contrast sensitivity the average monocular to binocular threshold was determined at all frequencies for each individual. The variations of monocular to binocular ratio with frequency were larger for our subjects than for those tested by Carpelletti (1966b) and Blake & Levinson (1977). However, average values for each subject showed much smaller fluctuations (Fig. 3). The arithmetic mean of the ratio for all spatial frequencies between 0.5 and 30 cycles/degree was  $1.09 \pm 0.05$  for the youngest,  $1.13 \pm 0.16$  for the middle and  $1.13 \pm 0.14$  for the oldest group. These values are almost the same as those obtained by Carpelletti (1966b) and Blake & Levinson (1977) for similarly aged subjects. In the individual and the group data we found a tendency for the ratio to increase with spatial frequency (Fig. 3).

At subthreshold contrast some subjects reported seeing a grating at a lower spatial frequency than that displayed. Most often they described a vertical pattern while the tested frequency could be of any value. Sometimes gratings of higher frequency were reported during the testing at a high frequency. No explanation for this phenomenon

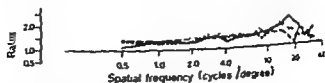


Fig. 3

The ratio of average monocular to binocular threshold for three age groups: 10–20 years, 20–40 years, and 40–60 years. Data points are the arithmetic mean of the ratio for all individuals in the age group.

## Discussion

findings are in close agreement with existing data with respect to the general shape of the contrast sensitivity curve. In all age groups the curve peaked in the frequency range of 3–5 cycles/degree and showed the typical attenuation in the low and high spatial frequency regions. Contrast threshold was quite easily defined even in the youngest subjects.

This study has shown that the contrast sensitivity is dependent on age. For subjects in our oldest age group (60–70 years) we found a decline in sensitivity to gratings in the middle and high spatial frequency regions that was not seen in younger persons. All subjects below 40 years of age had very similar contrast sensitivity curves. At ages between 40 and 60 years there was a gradual shift in contrast sensitivity towards the values of the old subjects.

The loss of sensitivity for high spatial frequencies found in the oldest persons is in agreement with the data of Arundale (1978). It might at least partially be explained as an effect of senile miosis (Woodhouse 1973). Our young subjects did not show the reduction in sensitivity to low spatial frequencies reported by Arundale (1978). It should be noted, however, that only three eyes of subjects in the group 8–13 years were tested by Arundale and that the mean Snellen acuity of these eyes was 0.8 while all our subjects had a visual acuity of 1.0 or more.

The difference between our young and old subjects might be considered quite small when compared to those reported in the literature between normal subjects and patients suffering from various visual disorders (see Introduction for references). However, we conclude that the age of the patient has to be taken into consideration when using the contrast sensitivity determination as a method of detecting visual dysfunction, especially in borderline cases.

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# CONJUNCTIVAL TRANSPORT OF TECHNETIUM 99<sup>m</sup> PERTECHNETATE

BY

T. SØRENSEN and F. TAAGEHØJ JENSEN

Using a gamma camera coupled to a computer and a tape unit the transport of pertechnetate ( $Tc^{99m}$ ) across the conjunctiva was determined by the region of interest technique in human subjects.

In 11 patients with their lacrimal sacs removed a fractional turnover rate of  $0.091 \text{ min}^{-1}$  was found. In 21 patients with inflamed conjunctiva due to chronic dacryocystitis a fractional turnover rate of  $0.094 \text{ min}^{-1}$  was found whereas the value in 11 normal individuals was  $0.017 \text{ min}^{-1}$ .

Noting the relatively small transcorneal route of disappearance the values found for technetium disappearance could be regarded as representative for the transconjunctival transport of the radioisotope. This assumption was confirmed by correlating the radioactivity in the blood with the fractional turnover rate.

*Keywords:* conjunctival transport - radioactive tracer gamma camera - human individuals - dacryocystitis

Little attention has been paid to the transport of solutes between the lacrimal sac and the blood across the conjunctiva. Though it is well known that atropine and other substances can influence other structures than the eye after instillation in the conjunctival sac due to absorption to the general circulation quantitative data on this subject are few.

It seems that Schirmer (1903) was the first to measure the disappearance of drugs from the conjunctival sac. He introduced salicylic acid into the eye of a patient with an excised lacrimal sac and found a five hundred fold decrease in concen-

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tration in  $1^25$  li which is equivalent to an 8.3% loss in concentration if no elimination is assumed.

In a study on permeability of excised bovine uvea (Sørensen & Tonboe 1977) it was found that the conjunctival-sclera combination was a selective barrier to certain substances as was the corneal-epithelium-stroma combination.

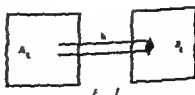
The first report on conjunctival transport using radiolabelled substances was by L'Esling (1967) who found a disappearance rate (fractional turnover rate) of  $0.001 \text{ min}^{-1}$  ( $0.0001 \text{ min}^{-1} \text{ cm}^{-2}$ ) in rabbits with closed canaliculi. The fractional turnover rate of  $^{125}\text{I}$  in an anesthetized animal was found when the cornea was protected by a suction cup.

Similar experiments have been carried out by Maurice (1973) on the permeability to sodium and chloride about ten times higher than  $^{125}\text{I}$ . The methods were not strictly comparable because Maurice forced a basin by pulling up the lids with clips—a procedure not used by L'Esling. The canaliculi surgically and left the eyelids untouched during the experiment.

In human beings the first results were presented by Meyer & Denffer (1971). They stated in a preliminary report that 30% of the technetium had disappeared from the conjunctival sac after 15 min corresponding to a fractional turnover rate of  $0.024 \text{ min}^{-1}$ . Similar measurements were presented at a meeting by Denffer & Dressler (1973). Their material comprised 11 patients with a block of the tear pathways. In these patients 23.2% of the technetium (pertechnetate in saline solution) had disappeared after 15 min, i.e. a fractional turnover rate of  $0.0176 \text{ min}^{-1}$ .

The present investigation was originally started in an attempt to establish background radiation in tear flow determinations (Sørensen & Tonboe 1973, 1977). However, it soon became evident that the transport of  $^{99}\text{Tc}$  could be surprisingly high.

In this paper the transport of technetium—as pertechnetate in saline solution—across the conjunctiva has been measured in patients suffering from dacryocystitis (group I), in patients with excised lacrimal canaliculi (group II) and in normal individuals (group III).



Model for calculation of fractional turnover rate.  $A_1$  is the activity in the compartment at time  $t$ ,  $A_2$  is the total activity in the body at time  $t$  and  $k$  is the rate constant for exchange between the compartments.

## Conjunctival Technetium Transport

2

radioactive tracer is introduced in the conjunctival sac in patients with or without the normal reflex tear outflow, the biological system can be simplified as shown in Fig. 1. Assuming exponential elimination from the conjunctival sac, the decrease of activity in the conjunctival sac follows equation (1)

$$A_t = A_0 e^{-\lambda t} \quad (1)$$

the increase of activity in the body equation (2)

$$B_t = A_0 (1 - e^{-\lambda t}) \quad (2)$$

where  $A_0$  and  $A_t$  are the radioactivity at time 0 and  $t$  respectively

$B_t$  the radioactivity at time  $t$  in the body

$e$  the base of the natural logarithm and

$\lambda$  the fractional turnover rate

transport of technetium from the general circulation to the tears has been considered so that it has been ignored in the calculation

## Material

study comprised 43 patients admitted to the hospital for dacryocystitis or for diagnostic purposes. The dacryocyst-exstirpated patients (group II) had had lacrimal sacs removed at least several months before the present investigation. Eyes of these patients were without inflammation. The normal individuals went through a tearflow determination as described in another paper (Sørensen & Taagehøj Jensen 1977). At the end of this determination no radioactivity had reached the nose. This is to be expected in some normal persons, since a primary test will only be positive in approximately 80% of normal eyes (Zappia & Her 1972). None of the normal volunteers suffered from watery eyes. With a point of interest including the conjunctival sac and the tear pathways, the appearance of radioactivity represented the transport to the blood. The normal subjects were younger (20–30 years of age) than the dacryocyst-exstirpated patients (60 years of age). The patients with dacryocystitis were 18–70 years old. The normal individuals were included in the normal material for tear flow studies (to be published).

## Method

1 detailed descriptions, dosimetric calculations, detection system and background radiation (Sørensen & Taagehøj Jensen 1977).

The radioactive tracer used was technetium  $Tc^{99m}$  as pertechnetate in a normal saline solution. The patient was placed in a supine position with the eye approximately 3 cm under

the pinhole collimator in the gamma camera. A volume of 10  $\mu$ l ( $^{99m}$ Tc) of the technetium solution was placed on the cornea while the lids were firmly held open. No anaesthesia was used.

Using the region of interest technique quantitative data of the elimination of activity from the designated area, i.e. the conjunctival sac in dacryocyst-exstirpated patients and the conjunctival sac and tear pathways in normals and patients with dacryocystitis.

The measuring time was 15 min. Radioactivity was accumulated at 10 sec intervals and the resulting 90 numbers were plotted as an activity time function curve in a semi-automatic system. The fractional turnover rate was calculated for the approximated curve in equation (1).

In these experiments the fractional turnover rate represented the amount of activity entering the general circulation per minute. Since the counting rates were very high a correction for background components was not necessary.

In 18 patients blood samples were taken 18 min after instillation in the conjunctiva. The uptake of technetium in the thyroid gland and gastric mucosa was blocked by per H<sub>2</sub>O 1 hour prior to the determination in some of the patients. In one patient the radioactivity in blood samples was measured at three min intervals after instillation.

## Results

In Fig. 2 a scintigram from a patient where the lacrimal sac has been removed. Fig. 3 is from a patient with dacryocystitis. To fill the lacrimal sac properly with technetium it was necessary to express the fluid retained in the sac prior to



Fig. 2



Fig. 3

Fig. 2  
Scintigrams of a patient with removed lacrimal sac. Most of the radioactivity is located in the inner and outer canthal area.

Fig. 3  
Scintigram of a patient with chronic dacryocystitis and retention in the lacrimal sac. Most of the radioactivity is located in this area. A similar picture was seen in the 11 normal individuals (see text).





Fig 4

Elimination curve from the conjunctival sac in a semilogarithmic plot. The fractional turnover rate is noted by  $k$ .

lation. In the 11 normal volunteers the scintigrams looked like Fig 3 with no activity in the lacrimal duct.

The elimination curve turned out to be a single straight line in the semilogarithmic plot in all the 43 patients (Fig 4). Thus the assumption of an exponential of elimination was acceptable.

The fractional turnover rates in the three groups can be seen in Table I. In groups I and III the absorptive area was the conjunctival sac, the cornea, the canaliculi and the lacrimal sac, whereas the absorptive area in group II was the conjunctival sac, the cornea and the canaliculi. Though the absorptive area was the same in groups I and III, the transport was significantly higher in group I than in group II. Patients with intermittent red eyes (Mann-Whitney test,  $2p = 0.0026$ ) and patients with a removed lacrimal sac also had a tendency to a higher transport of the blood compared with the normal individuals, though their absorptive area was a lacrimal sac less than that of the normal individuals (Mann-Whitney test).

Table I

Fractional turnover rate and the absorption of technetium after 15 min (calculated from the fractional turnover rate) in patients with a stop in the lacrimal passages at different levels and in normal persons

	Mean fractional turnover rate $\pm$ SEM	Fractional turnover rate converted to percentage absorption to the blood
Dacryocystitis $n = 21$	$0.0271 \pm 0.0036 \text{ min}^{-1}$	33%
Tear sac removed $n = 11$	$0.0214 \pm 0.0040 \text{ min}^{-1}$	28%
Normal individuals $n = 11$	$0.0145 \pm 0.0019 \text{ min}^{-1}$	20%

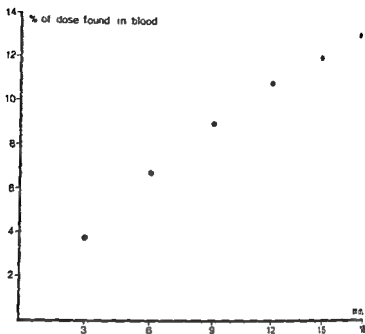


Fig 5

The increase of radioactivity in blood plotted versus time after instillation in the conjunctival sac in one person

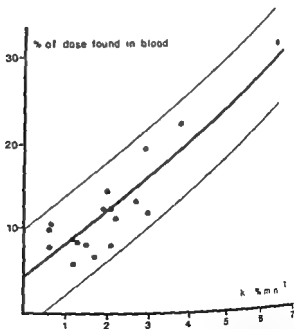


Fig 6

Correlation between the amount of technetium found in the blood 18 min after instillation and the fractional turnover rate in 18 persons ( $k$  in the figure)

> 0.10). These normal volunteers did not have a history of watery eyes and they had normal diaphasic curves without any sign of obstruction of the tear pathways. The fractional turnover rate in groups I and II were not significantly different ( $t = 0.2-0.3$ ).

A confirmation of the transport to the blood is given in Fig. 5. The values in the blood were not corrected for the leaking of technetium out of the general circulation since the magnitude of this transport is relatively unknown in a short time situation like this. With this inaccuracy in mind a rough calculation of the rate increase in the blood resulted in a value of the same size as the fractional turnover rate in this patient.

In Fig. 6 the correlation between the fractional turnover rate and the amount of technetium found in the blood 18 min after instillation is shown. One of these terminations was discarded because for some unknown reason it was more than two standard deviations from the regression line. There was no difference between the patients premedicated with perchlorate and those with no premedication. In this correlation study patients from all the three groups (Table I) were included.

## Discussion

Although it has been known for many years that drugs introduced in the conjunctival sac can reach the general circulation, most studies on the transport rates in the human eye have been concerned on the permeability of the cornea. Until the works of Ursing (1967) and Maurice (1973) only semiquantitative data on conjunctival permeability were available. Unfortunately, Maurice found values ten times higher than Ursing, probably owing to the greater absorptive area in Maurice's rabbits with a conjunctival basin formed by pulling the lids. Thus, Ursing's method seems most comparable to the *in vivo* conditions found in man. When the fractional turnover rate in Ursing's paper is corrected for the bigger surface area in man, a value of  $0.006 \text{ min}^{-1}$  for the positively loaded ion  $\text{Na}^{22}$  compared to  $0.021 \text{ min}^{-1}$  found in our work for the negatively loaded pertechnetate. Denffer & Dressler (1976) found a value of  $0.018 \text{ min}^{-1}$  in 68 patients with stop in their tear passages. Our result in a comparable group of patients was  $0.025 \text{ min}^{-1}$  ( $s = 0.015$ ). Mean of group I and II (Table I) corresponding to an absorption of technetium to the circulation of 31% in 15 min (Denffer & Dressler 23%). This difference can probably be explained by dissimilarities in methods. In a meeting programme Denffer & Dressler (1976) demonstrated a tendency to a greater absorption the higher the stenosis in the tear pathways. We found the same tendency with higher fractional turnover rates in patients with removed lacrimal sac and dacryocystitis patients than in normal

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## TIMOLOL MALEATE IN TREATMENT OF OPEN ANGLE GLAUCOMA

BY

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The effect of Timolol maleate on the intraocular pressure in open angle glaucoma has been examined in 37 patients of these simple glaucoma in 26 (48 eyes) capsular glaucoma in 9 (14 eyes) and chronic secondary glaucoma in 2 patients (2 eyes). These cases constituted a group which was relatively difficult to manage.

The average pressure reduction caused by Timolol maleate alone was about 24%. In 14 patients the intraocular pressure was adequately controlled on Timolol as the only drug and in 10 on additional drug therapy. Five patients failed on drug treatment and the remaining four failed on one eye while the fellow eye was well regulated.

Tonography indicates that the effect is caused by a reduction of the aqueous humour production.

Side effects of locally applied Timolol maleate have not been observed. This drug may be the drug of choice in many instances.

**Key words:** Timolol maleate - beta adrenergic blocking agents - open angle glaucoma - simple glaucoma - capsular glaucoma

Over the course of the last few years several beta adrenergic blocking agents have been introduced in glaucoma treatment. Both oral and local application have been proved to reduce the intraocular pressure. Such drugs have included atenolol (Brenkman 1976, Wettrell & Pandolfi 1977, Philips et al 1977), propranolol (Wettrell et al 1967, Cote & Drance 1968, Musini et al 1971, Vale et al 1970, Wettrell & Pandolfi 1975, Borthne 1976), bupranolol (Kneegstein et al 1974), pindolol

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omi & Steindler 1975) oxprenolol (Stilma 1976) and Timolol (Katz et al 1976, Jilles et al 1977, Zimmerman & Kaufman 1977, Zimmerman et al 1977, Jensen 1978, Kerty & Hørvén 1978). Among these Timolol maleate seem to have advantages over the others. Applied locally the cornea sensibility is unaltered in contrast to propranolol and bupranolol and the effect on the intraocular pressure lasts considerable longer than after application of atenolol, pindolol or enolol. In addition Timolol maleate has been used systemically for several years in the treatment of arterial hypertension and angina pectoris without reports of serious side effects.

In the last two years clinical trials using Timolol maleate have been initiated over many areas of the world. The intention with this paper is to report our experience with locally applied Timolol in the treatment of various types of open angle glaucoma. Of special interest is the effect on capsular glaucoma.

## Material and Methods

The clinical trial comprised 37 patients with open angle glaucoma where simple glaucoma was found in 26 (48 eyes), capsular glaucoma in 9 (14 eyes) and 2 patients (eyes) had chronic secondary glaucoma caused by chronic uveitis in one and congenital cataract treated surgically in the other. Fourteen were inpatients and 23 outpatients.

The one patient with operated congenital cataract and secondary glaucoma was 6 years-old while the age distribution of the remainder of the patients ranged from 46 to 82 years. Two patients were new cases (1 simple and 1 capsular glaucoma) while the others had previously received conventional antiglaucomatous therapy.

**Preoperative procedure.** Prior to the trial all glaucoma medication was stopped. After a "wash-out period" varying from one to seven days the following examinations were performed: Ordinary ophthalmological status including perimetry, pupillometry, Goldmann tonometry (Goldmann tonometer), evaluation of tear production by Schirmer test and pulse and blood pressure measurements. These examinations were repeated during the control period.

The aim was to establish an intraocular pressure (IOP) below 22 mm Hg. Initially Timolol 0.25% once a day (at night) was given. Depending on the IOP level seen at the end of the wash out period the pressure was controlled one to seven days later. If the IOP still exceeded 22 mm Hg the Timolol medication was increased to 0.5% once a day and later to 0.5% twice daily if necessary. If the IOP still exceeded 22 mm

satisfactory pressure regulation. Another patient (No 9) had good regulation of simple glaucoma in his left eye on Timolol medication for 3 months, followed by a dramatic rise in pressure refractile to any medication and trabeculectomy was carried out. His right eye with secondary glaucoma caused by uveitis was however well maintained on Timolol alone as illustrated in Fig 1. These patients (Nos 11 & 9) are registered in the column for iop above 22 mm Hg in Table I.

The effect of Timolol and additional therapy in 17 patients is seen in Fig 2. In these patients Timolol alone was not able to maintain the iop below 22 mm Hg. In two patients (Nos 7 & 23) the iop was even higher on Timolol alone than during the wash out period.

In 10 of the patients the iop was well regulated on Timolol and additional therapy. Drug therapy has failed in 7 patients and in 6 of them a trabeculectomy was performed (patients Nos 6, 12, 22, 23, 30 and 31). Three of these had satisfactory tension levels in the fellow non-operated eye using drug therapy as illustrated in Fig 2 (patients Nos 6, 22 and 31).

Table II shows the comparison between the therapeutic response to the prior therapy and to the present therapy. Out of 19 patients who could be well regulated on Timolol alone, 13 had previously used two local drugs and another 4 had also

Table II  
Comparison of prior therapy and present medication.

PRIOR THERAPY	NO PAT	PRESENT THERAPY WITH I O P < 22 mm Hg				LOCAL DRUGS ACETAZOL.	I O P > 22 mm Hg IN DRUGS
		NO PAT TIMOLOL	NO PAT TIMOLOL EPINEPHR	NO PAT TIMOLOL PILO	NO PAT TIMOLOL PILO		
NONE NEW CASES	2	1				1	
ONE LOCAL DRUG (PILO ? EPI ?)	8	5	1				
TWO LOCAL DRUGS (PILO EPI)	9	6	1				
LOCAL DRUGS							
ACETAZOLAMIDE	17	4	1	1		4	
ACETAZOLAMIDE	1					1	
TOTAL	37	11	2	1		6	1
I O P > 22 mm Hg ON PRIOR THERAPY AMONG THE 37 PATIENTS	16	6	2	0		2	1

# Timolol in Open Angle Glaucoma

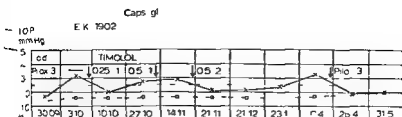


Fig 3

ire curve from a patient showing a relative slowly developing insensitivity to Timolol reverting to normal level when converted to pilocarpine

ed acetazolamide. Among the 18 patients who had previously used acetazolamide six had no need for this drug while on the present therapy. On prior py 34 patients had to use pilocarpine 3 times a day and/or acetazolamide of these patients were well regulated on Timolol alone with application one (14 nts) or two (3 patients) times a day. Among the 9 patients with iop above 22 Hg drug treatment failed on one eye in 4 patients while the glaucoma in the eye was well regulated.

it of 16 patients who with previous medication had iop above 22 mm Hg 10 nts were well regulated by the new treatment and 6 of these with Timolol.

The average reduction of iop in the total group was about 23% with standard ation 16.4.

the effect of Timolol maleate could be registered already 9 respectively 15 h the first application. Some patients however subsequently developed relative insensitivity to the drug requiring a stronger solution of Timolol or the addition of additional drugs. Such relative insensitivity was already observed in one patient on the first week of treatment and in another case during the first 5 to 6 nts (Fig 3).

onography has shown almost no change in the facility of outflow not even when iop dropped from 46 to 16 mm Hg from one day to the next.

## effect of Timolol on different types of glaucoma

figs 1 & 2 it is shown that only one of the 9 patients with capsular glaucoma could be maintained on Timolol as the sole drug while 17 of the 26 patients with simple glaucoma required Timolol medication alone. Surgical treatment had to be performed in 4 patients with capsular glaucoma (patients Nos 11, 23, 30 and 31). The trabeculectomy was performed in only 2 patients with simple glaucoma (patients Nos 12 & 22).

The average reduction of the IOP on medical treatment was approximately 50% in 11 eyes with capsular glaucoma and approximately 26% in 18 eyes with glaucoma.

The two patients with chronic secondary glaucoma (patients Nos. 9 & 11) had satisfactory pressure levels on the new regimen and one of these required no Timolol to maintain this. Both these patients had previously needed more treatment and one of them was even then not adequately controlled.

**Side effects** Two of the patients showed at one visit a pulse rate below 60 per min but had no subjective symptoms and the frequency was easily increased by light exercise. No influence on blood pressure was recorded. During treatment with Timolol as the only drug, the pupillary diameter has been about the same as during the wash-out period and the pupillary reactions have been normal. We have seen no changes in the conjunctiva, cornea, lens or retina. No progressive optic nerve damage have been observed. We have not recorded any lowering of tear production as judged by the Schirmer test.

## Discussion

This study supports the earlier reports that Timolol maleate has a marked pressure-reducing effect when applied locally to open angle glaucoma. The mode of action seems to be a lowering of aqueous humour production with no alteration of the outflow facility. This agrees with earlier observations (Zimmerman et al. 1977). The present study is an open study, not randomized, and comprises a relatively high proportion of glaucomas difficult to treat. Thus 14 patients have been admitted to the department and 11 patients had glaucoma of such a severity that surgery was considered the only alternative. It is worth noting that 3 of these 11 were satisfactorily regulated on the new medical therapy while the remaining 8 had to undergo surgical treatment. Among the 37 patients 18 were maintained satisfactorily on Timolol alone while the remainder needed the addition of one or more additional drugs (13 patients) or surgery (6 patients).

The effect of Timolol is seen by a decrease of the IOP the day after the first application. The relative insensitivity to the drug seen in some patients is probably explained by an initial dramatic drop of aqueous production with a later stabilization at a higher level.

Our study indicates that capsular glaucoma responds less well to Timolol than the simple glaucomas as 60% of the patients with simple glaucoma required Timolol as the only form of treatment compared to 11% of the capsular glaucoma.



gust the 16 patients with iop exceeding 22 mm Hg on previous treatment 3 capsular glaucoma and all these had iop exceeding 22 mm Hg at least in one eye on present medication. This confirms the earlier observation that capsular glaucoma is a more difficult type of glaucoma to treat than simple glaucoma (Hertel 1971, Kertu & Horven 1978).

Applied locally Timolol maleate has a prolonged effect. Out of the 18 patients treated on Timolol as the only drug, 15 needed only one application a day. As the interval between application and iop registration was 15 h for the outpatients and 19 h for the inpatients, this study supports earlier investigations that the effect of Timolol lasts longer than 24 h (Zimmerman et al. 1977).

This study also confirms the absence of side-effects using this drug. This has been previously expressed by many patients who enjoy better sight and night vision. Symptoms of earlier treatment have disappeared. The reduction of applications 3 times a day must also be of great benefit.

In this investigation the pressure reducing effect of Timolol in most cases has almost unchanged during the period of observation. If the pressure reducing effect of Timolol maleate proves to be a lasting one over several years of treatment without any serious side effects this might well represent the beginning of a new era in the treatment of open angle glaucoma.

Our present experience indicates that Timolol maleate in many instances will be the drug of choice.

### Acknowledgment

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# CAVERNOUS HAEMANGIOMA OF THE RETINA

BY

INGIMUNDUR GISLASON STAFFAN STENKULA ALBERT ALM  
EIVIND WOLD and PER ERIK WÄLINDER

Seven cases of retinal cavernous haemangioma are presented. Three cases have been followed for more than 11 years and three cases between 1 and 2 years. Six cases had no eye symptoms related to the vascular tumour while in one case vitreous haemorrhage occurred on two occasions. On both these occasions full vision was regained. None of the vascular tumours were treated. Two patients had grand mal seizures. They also had convulsive disease in the family history. In three cases family members of two generations were found to have normal eyes on examination.

*Key words:* Cavernous haemangioma — retina — vascular disturbances — epilepsy

Cavernous haemangioma of the retina and optic disc is a specific vascular tumour. It is supposed to be inherited in an autosomal dominant mode and be associated with similar lesions of the skin, brain and other organs (Gass). The tumour consists of a group of round dark red aneurysms sometimes covered in white fibrous like tissue. Fluorescein angiography demonstrates delayed perfusion and a characteristic appearance of plasma erythrocyte layering in the aneurysms. Leakage of the dye does not occur. Haemorrhages from this vascular malformation are rare and exsudation has never been reported. Wess et al. (1975) published three cases and presented a review of 35 cases.

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previously reported in the literature Klein et al (1975) reported 41 cases (11/75) and Winning (1976) presented one case each Patton et al (1976) showed two cases in their atlas Colward et al (1978) reported 2 cases associated with myoclonic features

In this report we describe 7 cases examined in Sweden during the period 1975-1978

## Case Reports

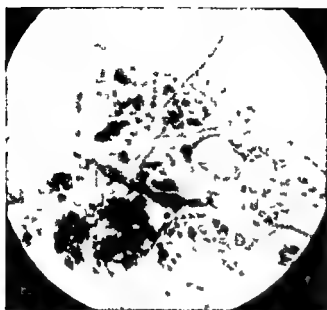
### Case 1

Female born in 1940 The patient had suffered from intermittent pains in the left side of the face for many years but was otherwise in good health A 16-year-old sister died from a subarachnoid haemorrhage of unknown cause The patient had a severe mucous haemorrhage in 1968



Fig 1

Case 1 Large aneurysms in the periphery of the right fundus Enlargement of the upper aneurysm



*Fig 2*

2 A nasal sector of the left fundus. Aneurysms of different sizes. Some of the largest appear to be fibrotic.

After bleeding in 1946. On both occasions she regained full vision. After the first bleeding a grey fibrous mass with cystic thin-walled formations was found in the temporal periphery of the fundus. Several large cysts embedded in the mass were filled with dark blood, which showed the typical sedimentation of large cavernous haemangiomas (Fig 1). The other eye was normal. There seemed to be no change in the lesion at examinations up to

2

born in 1939, an immigrant from Yugoslavia. The family history was unremarkable. He was in good health and had never had any eye symptoms. When he was treated for a corneal injury in 1966 a retinal vascular malformation was found in the periphery of the left eye (Fig 2). It consisted of round grape-like aneurysms filled with blood in a grey fibrous mass of tissue. Otherwise the eyes were normal. The patient was not available for further examinations.

3

born in 1943. His father had epilepsy and a son was mentally retarded and had epilepsy. In 1963 a vascular skin tumour was removed from his leg. Pathological examination showed a haemangioma with traumatic ulcerations. In 1969 he had an ophthalmological examination after two incidents of grand mal convulsions. His vision was normal and he had no other symptoms. In both fundi clusters of saccular aneurysms were found in the periphery.

3) Otherwise the eyes were normal. Pneumoencephalography and cerebral angio-

graphy were normal. Subsequent examinations have not revealed any changes in the fundus. Fluorescein angiography in 1977 showed the typical picture of cavernous haemangioma, i.e. slow perfusion and plasma erythrocytic sedimentation. The patient's son was normal on eye examination.

#### Case 4

Male, born in 1956. The family history was negative. In 1959 he was found to have poor vision with myopic correction. In the periphery of the right fundus there were tumour formations. The tumour was encircled by a small rim of fibrous like tissue. The left eye was normal. He had no history of vitreous bleedings or convulsive disease. He was reexamined in 1978. When photographs from this examination were compared with those from 1972, it was found that some of the aneurysms had been replaced by fi-

#### Case 5

Male, born in 1907. His medical history includes bronchial asthma, heart disease and an operation for a thyroid adenoma. He had one attack of grand mal in 1969 and in 1977. There was no family history of ocular diseases. An uncle had convulsions, a son had epileptiform attacks, thought to be caused by a previous slight head injury. Ophthalmological examination after the grand mal attacks in 1977 revealed dilated vascular aneurysms in the retina of the left eye. The visual acuity was 0.4. Fluorescein angiography showed a slow perfusion and plasma erythrocytic lumen phase (Fig. 4). Neurological examination including computed tomography was normal. He had no vascular skin tumours. Four of the patient's children, including the son with convulsive disease, were examined and they were found to have normal eyes.

#### Case 6

Male, born in 1966. He was in good health. The family history was negative. In 1966 he was examined for astigmatism. The corrected visual acuity was 0.4 in the right eye and 1.0 in the left eye. In the right fundus there was a vascular wedge shaped tumour in the periphery. It consisted of a protruding dark blue mass of dilated vessels filled with blood. Fluorescein angiography confirmed the diagnosis of cavernous haemangioma (Fig. 5). The left eye was normal. On reexamination in 1978 the eye tumour was unchanged. His parents, a sister and a brother were examined and had no eye diseases.

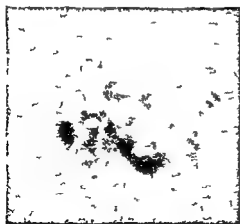
#### Case 7

Female, born in 1973. The family history was negative. On routine examination at 13 months of age a large vascular tumour was discovered in the nasal peripheral part of the right fundus (Fig. 6). The tumour was built of round, dark blue saccules, some of them were surrounded by white or grey tissue. Fine retinal folds extended from the tumour on optic disc. Other parts of the fundus and the other eye were normal. She had full vision and had never had any convulsive symptoms. Neurological and paediatric examination were normal. No haemangiomas were found in the skin. Reexamination in October 1978 showed no changes of the tumour. Her parents and only sister were found to have normal eyes.



*Fig 3*

Case 3 Right eye with a cavernous haemangioma Lattice degeneration in the periphery



*Fig 4*

3 Left eye Fluorescent angiogram Erythrocytic sedimentation in the late phase of the fluorescein angiogram



*Fig. 5*

ase 6 Fluorescein angiogram right eye Arterio-venous phase Notice erythema (arrow) and delayed drainage in one of the veins (arrows)





*Fig 6*

*Fig 7* Right fundus. A big vascular tumour in the nasal periphery. Stretching of vessels towards the lesion

## Discussion

Most of the patients in our material have been followed during periods of different length from 1 to 22 years. We have not found any progress of the lesions in any of these cases. Krause (1971) reported a case which had been observed for 13 years and showed slight progress. Lewis et al (1975) reported no progress in a case observed during 34 years. In one of our patients the lesion showed increased fibrotic changes (case 4).

These tumours seldom give symptoms. One of our cases had two episodes of vitreous haemorrhages but regained full vision. Three patients have previously been reported to have bilateral lesions (Scheving 1937, Reese 1963, Frenkel & Reese 1967). One of our patients had retinal haemangiomas in both eyes (case 3). It



*Fig. 5*

Case 6 Fluorescein angiogram right eye Arterio-venous phase Notice erythroretinopathy and delayed drainage in one of the veins (arrow)

*Cavernous Haemangioma of the Retina*

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## THE PALLOR OF THE OPTIC DISC

A quantitative photographic assessment by purple light

BY

PER NELLEMANN SØRENSEN

By employing purple colour filters with a large blue and a small transmission normal and atrophic optic disks were photographed on transparencies. Normal optic disks reflect red light and atrophic reflect blue light. The degree of atrophy estimated by colour quantitated by microdensitometry in the blue (410 nm) and in the red region of the transparencies. The difference between the blue and red is used as an expression of optic disc colour.

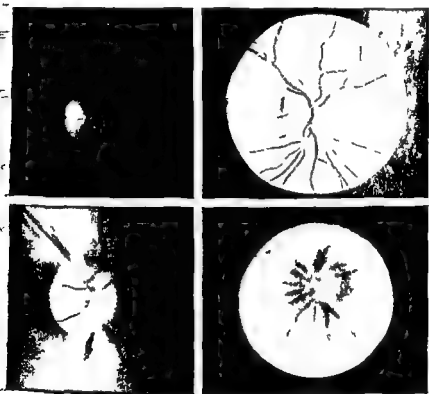
**Key words:** microdensitometry - optic atrophy - colour contrast - interference filter - purple filter - photography - quantitative measurement - optic ophthalmoscopy

The degree of pallor of the optic disc is considered a means of evaluating atrophy. The colour of the normal disc is due to vascularization since tissue is grey (Walsh & Hoyt 1969). When the optic nerve fiber bundles a white reflecting glial tissue is formed on the optic disc the vascularization decreases ophthalmoscopically and a corresponding pallor of the disc is seen (Quigley & Anderson 1977).

The purpose of this paper is to introduce a qualitative or quantitative photographic method of assessment of the pallor of the optic disc by means of a fixed interference filter in the light path. The filter transmits a large amount of blue light and a small amount of red light. The blue light is easily absorbed by the haemoglobin in the vessels and scattered to a lesser degree whereas the red light is primarily reflected from the haemoglobin giving rise to the red impression of

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ization often on a blue contrasting background. Since the overall transmission of the filter is blue, the colour impression of the disc is blue when the disc is almost completely avascular. On the other hand, when the vascularization is only decreased, a mixture of red and blue is seen, and this purple colour is easier to define than shades of red alone. In his method, allowance is made for variations in exposure and processing; colour photography is performed.



*Fig 1-4*

- 1 Temporal pallor of the optic disc due to multiple sclerosis photographed with purple filter. The blue colour denotes atrophy and red normal tissue (Flash 300 Wsec. Kodachrome 25)  $\times 10$
- 2 Temporal pallor of the optic disc photographed without filter (the same patient as in 1) (Flash 180 Wsec. Kodachrome 25)  $\times 10$
- 3 A normal optic disc photographed with purple filter. The red optic disc contrasts with the blue retina.  $\times 10$
- 4 A glaucomatous optic disc photographed with purple filter. The atrophy is seen as a blue colour.  $\times 10$

## Methods

Photographs are taken with a standard Zeiss (Oberkochen) fundus camera. The filter used is of a multilayer interference type which is used for colour fluorescence photography and described by Jensen & Olsen (1974). This filter has a 80 per cent blue transmittance between 425–500 nm and a narrow red band at 640 nm with a half band width of 8 nm, transmittance 50 per cent.

Kodak Wratten 32 (Magenta) alone or in combination with Wratten 59 which is standard in the Kowa and Topcon fundus cameras were also tested but the red transmittance was found to be too high.

Pictures were taken with the pupils dilated with colour disposable films of subtractive primary colour coupled (Agfachrome professional 30 S (3000 Å) or Ektachrome 16 and Fuji 16) which had their colour laid on during the development (Kodachrome 23 and 64).

Pictures were taken with and without filters at varied exposures by variation of the vessel flash intensity which was regulated via the charging voltage.

The Ektachrome films were developed by a private laboratory by process E-4 the same as for the films by the manufacturers.

A variation due to change in the light sensitive material by time or manufacturing variation due to change in development was checked by photographing a minimum of a paper copy of the star shaped Kodak enlarging negative on each film.

According to information provided by the manufacturers there is only a small overlapping of sensitivity between the cyan and yellow forming layers of the films which makes it possible to respectively blue colours on the final transparency. A sensitometric curve was constructed by densitometry employing a microdensitometer consisting of a Reichert microdensitometer built in Schott verlauffilter with 10 nm half band width. The density was read at 400 and 640 nm corresponding to the maximal transmittance of the blue and red colour. The resulting curve was linear with a correlation coefficient  $R^2 > 0.95$  for both blue and red. The slope varied less than 5 per cent between blue and red in agreement with the sensitometric data from the manufacturers (from Fuji no data were obtained).

On the optic disc pictures the density was read on the vascular rim below the superior temporal vessels. The measuring area correspond to 50  $\mu$  in the emmetropic subject. The difference between the densities at 400 and 640 nm. Dens-Dens was taken as an index of the colour balance between blue and red. Thus variations in the exposure and processing of the subtractive films affects the densities in the same direction.

## Results

Examples of the blue and red filter technique are seen in Figs 1, 3 & 4.

Figs 1 & 2 demonstrate the difference between a fundus photograph of a patient with temporal pallor of the optic disc in white light (120 W/sec Kodachrome 23) and with the blue/red filter (350 W/sec). The patient is a 91 year-old woman with multiple sclerosis which has caused a central scotoma with vision decreased to 6/18.

# The Pallor of the Optic Disc

Table I

ifference between the densities at 470 nm and 640 nm on the vascular rim of optic disc filter photographs. Five films are compared in two patients with optic atrophy in one and a normal fellow eye. The mean density of two photographs in each eye is given.

	Film Speed (ASA)	Flash Wsec	Optic disc $D_{470}-D_{640}$			
			Normal		Atrophic	
			I	II	I	II
Kodachrome 35S	63	240	0.92	1.02	0.62	0.72
Kodachrome V	63	240	0.84	0.76	0.62	0.58
Kodachrome 25	25	950	1.52	1.62	0.52	0.05
Kodachrome 63	63	940	2.35	1.94	1.05	1.00
	100	180	0.98	0.84	0.68	0.2

Table I demonstrates that Kodachrome 25 film gave the greatest difference between a red vascularized area and a pale atrophic area of the optic disc. The colour balance of the optic photographs: the amount of blue and red is expressed as the difference between the densities at 470 and 640 nm. Thus a small value gives a bluish hue and a high value a reddish hue.

Table II demonstrates that only at flash intensities above 240 Wsec the  $D_{470}-D_{640}$  is constant and a linearity exists between film density and exposure for blue and red light. Below 240 Wsec the density at 640 nm exceeded 3.0 and thus the proper exposure is passed as found by the exposure card.

Measurements on transparencies with the optic disc pictured in the periphery or sharp pictures gave a bluish false balance.

Table II

Blue and red colour balance of normal ( $n = 3$ ) and atrophic ( $n = 3$ ) optic discs at varied exposures on Kodachrome 25 transparencies and with purple filter.

Flash intensity (Wsec)		120	180	240	350
Optic disc $D_{470}-D_{640}$	Normal	0.05	0.72	1.40	1.45
	Atrophic	-0.15	-0.28	-0.31	-0.32

## Discussion

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With the described purple (blue/red) filter technique for estimation of the colour of the optic disc difficulties lie in the control of exposure i.e. the illumination of the fundus of the eye which may vary with changes in the optical media and the angle of illumination due to small eye movements and variation in film and development. With the filter technique changes in the spectral light distribution due to changes in the glow lamp intensity or type of lamp are decreased and they can be avoided by means of bluish conversion filters.

Changes in the optical media are usually due to increased light absorption by the ageing lens of the patient and observer (Miller & Benedek 1973). Since an apparent increase in red colour of more than 30 per cent between the ages 0-60 years (Said & Werle 1959; Boettner & Wolter 1962) a correction for the age factor is necessary. The change due to variations in exposure is accounted for by the use of two measurements at different wavelengths at only one spot on the optic disc.

An error due to different sensitivity of the blue and red sensing material is minimal as seen from the sensitometric curves of the blue and red sensing material. Furthermore the error is multiplicative and not additive.

Schwartz (1976) has solved the problem of variation due to changes in film intensity and film development by photographing a step wedge simultaneously with the fundus. However this error appears to be small compared to changes in the optical media (Sørensen 1977).

The measurement of colour contrast for estimating the degree of pallor of the optic disc has also been studied by Bock (1950), Gloster (1969), Berkowicz & Bock (1970), Davies (1970) and Gloster & Parry (1974).

By using polychromatic film we have adopted the ideas of Gloster (1969) and Davies (1970) who measured the green and red density of white light and transparencies for obtaining a measure of vascularity from the reflection of haemoglobin. But we have also incorporated the old idea of using red filters (Cullstrand 1906; Vogt 1918) by using a filter with a dominantly blue transmission.

Thus these and our methods rest on the assumption that pallor of the optic disc is synonymous with avascularity but pallor may represent tissue in which rearrangement of astrocytes in dense parallel layers has taken place resulting in altered light scattering and reflecting (Quigley & Anderson 1971). The fluorescence of the optic disc in fluorescein angiography in normals does not correlate with the ophthalmoscopic observations (Hayreh 1969, 1971). However when optic atrophy is present (O'Day et al. 1967; Hayreh 1969) the fluorescence is generally reduced. Also with patent blue V deep red absorption at wavelengths 640 nm the optic disc is even filled in normals (Sørensen 1977).

Gloster & Parry (1974) in their cyan filter methods for estimation of the



matous cupping and pallor point to the possibility that at least a part of the red light from the optic disc comes from light diffused into the optic disc rim from the choroid. This problem is of greater importance in evaluating the pallor of an atrophic optic disc with a narrow vascular rim, but slit lamp examination using contact lens shows that the optic disc generally first begins to glow faintly when the slit reaches the optic disc border and then the entire disc glows due to its translucency (own observation). In all the described purple filter with its potential of quantitative technique may be an improvement on the common red free filter technique for the detection of optic disc pallor by colour contrast.

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## BIOPLAST® FIBRIN FILM FOR CONJUNCTIVAL REPLACEMENT

BY

ISTVAN TAPASZTO and GEZA KERENYI

The study includes 43 cases of conjunctival grafting in chemical burns and traumatic pterygium. Resorbable Bioplast® fibrin film was used as a readily available biocompatible conjunctival substitute. The implant was absorbed and the site occupied by fresh conjunctival tissue in a few weeks. The composition of tear proteins was restored to normal as fast as after free conjunctival grafting. The results were also satisfactory in terms of cosmetics.

**Keywords:** Bioplast® – fibrin – conjunctival replacement – biocompatibility

Industrialization has led to an increasing occurrence of eye injuries. Severe acid and alkali burns may necrose large areas of the conjunctiva and result in corneal opacity and disturbing scars.

For the immediate chemotherapeutic measures, the treatment of these injuries is based on an operation for replacing the necrosed conjunctiva. Literature is abundant in reports on the use of various conjunctival grafts. The materials tested include canine conjunctiva (Wolfe 1873), human conjunctiva (De Wecker 1879), conjunctiva and vaginal mucosa (Stellwag von Carion 1889), amnion (de Tott 1940), conjunctiva (Malhotra 1957), prepuce (Soliman 1969), silicone rubber (Merz & Winkler 1969), etc. The variety of these materials shows that none is sufficiently suitable to find general acceptance although human conjunctiva (Gundersen flap), conjunctiva and nasal mucosa are quite suitable in many situations. The major disadvantages of conjunctival substitutes are limited availability, difficulty of storage, postoperative atrophy, unsatisfactory cosmetics, etc.

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A conjunctival autograft is certainly the best solution but its use is not feasible in injuries extending over large areas of one or both eyes. In most cases, but mucosal grafts are applied as a substitute for excised tissue.

Our objective was to test Bioplast fibrin films for this purpose and to see if the method compares favourably to currently applied techniques.

## Material

### Patient material

The study comprises a series of 43 cases of conjunctival grafting with Bioplast in 32 patients (25 males 7 females). Of the 43 injuries 11 were fresh chemical 12 burns occurred earlier and resulted in scar formation and symblepharon and were 20 cases of traumatic pterygium. Most injuries were caused by lime or by lesions studied over a period of 4 years were too extensive to be solved by grafting with autogenous conjunctiva.

A control group included 6 alkali burnt patients (4 males, 2 females) treated with free transplantation of conjunctiva from the other eye and 4 burnt patients (6 males 2 females 10 eyes) grafted with oral mucous membrane.

The follow up time amounted to at least 4 years after the latest operation in the experimental series and in the control group.

### Implant material

Bioplast is a biocompatible absorbable implant material made from washed bovine fibrin. Endoprostheses are prepared by compression moulding a mixture of powder, glycerine and water (Gerendas 1960).

The resorption time of the implants can be modified by crosslinking with formalin (Gerendas 1968).

No infection, fever or undue histological reaction follows implantation (Gerendas 1968). No clinical symptoms of an immunological response can be detected even after the implantation of the heat deantigenized bovine material (Horvath et al. 1965).

Resorption proceeds at an even rate and metabolites are eliminated mainly while the site is gradually invaded by connective tissue (Torok et al. 1965).

Buttons were applied for the protection of liver sutures (Wood et al. 1961) and reported as useful aids for scleral buckling in retinal detachment (Crosby et al. 1973). We have been successfully applying films for skin replacement in ophthalmology (Tapasztó et al. 1977). Bean shaped implants were used in female urinary stress incontinence (Hartmann 1975).

Other future applications of Bioplast implants have been suggested (Carter et al. 1977).

The first industrially produced Bioplast implants were put on the market in Europe in autumn 1976. They are sold under the registered name Biethum.

## *Fibrin for Conjunctival Replacement*

Bioplast films used for conjunctival grafting were prepared from human fibrin. They were 10 × 10 or 20 × 20 mm in size and 0.2 mm in thickness. They were soft and easy to handle due to their high glycerine content. They were moderately crosslinked and had a shelf life of 2–3 weeks. The sterile Bioplast films were obtained as materials for clinical

### Method

**Preoperative preparation**  
Surgery for recent injuries is scheduled as soon as possible since these cases are more amenable to treatment. Preoperative preparation includes irrigation of the eye with saline, instillation of adrenaline (Suprarenin, Epinephrine) and cocaine, followed by excision of the affected conjunctiva in local anesthesia (0.001% adrenaline + 2% cocaine). In older injuries with symblepharon, the scarred conjunctiva is excised and when a symblepharon is present, the tarsal plates of the bulb and eyelids are separated. After excising the necrotic tissue, the graft is placed on normal tissue where bleeding occurs. conjunctival replacement is performed by the thin Bioplast fibrin film of which a piece is cut corresponding to the size of the missing conjunctiva (no contraction of the implant was observed). The film is held in place with 7–0 silk black braided Ethicon sutures. It is anchored to the host conjunctival edges so that the margin of the graft is covered by the host tissue. An antibiotic ointment (Terramycin and Neomycin) is then placed in the conjunctival area and the eye is bandaged. The dressings are changed twice a day. Sutures are removed 6 days postoperatively. Systemic antibiotic administration is discontinued 10 days postoperatively.

**Chemical methods**  
In addition to studying the clinical efficiency of Bioplast fibrin grafts, a comparison of the biocompatibility of free conjunctival and buccal mucosal transplants and Bioplast was performed. Postoperative tear protein content and composition were determined to this end. The methods were reported elsewhere (Tapaszto 1974).

### Results

**Clinical results**  
The clinical course was reassuring in all 43 cases. A good take of the graft was always seen. Pronounced exudation was seen on the first few days following transplantation. The implant was initially transparent and yellowish, then became white, exhibiting the fibrin network. No more discharge was seen after suture removal 7 days postoperatively. At this time, Bioplast still appeared as a whitish substance, but the beginning of vascularization was already observed along the

edges. No shrinkage of the implant was seen (Fig. 1). Fifteen days after implantation the Bioplast film had been transformed into or replaced by conjunctiva. The cornea was covered by fresh tissue which did not differ from the surrounding cornea (Fig. 2).

Of the 11 fresh chemical burns, slight symblepharon formation was noted in 4 cases at the follow up 1 year postoperatively. They were definitively removed by repeated Bioplast implantation. Of the scar and symblepharon cases from earlier burns, 3 symblepharons needed reoperation, while of the 29 cases of traumatic pterygium, 5 had to be reoperated once.

*Fig. 1*  
Traumatic pterygium on the right eye 7 days after Bioplast implantation.



*Fig 2*

The eye shown in Fig 1 13 days postoperatively

In the control group of 6 cases treated with conjunctival transplantation from the other eye 2 had to be reoperated. Of the 4 control cases where oral mucosa was transplanted 3 cases required one reoperation.

The situation achieved by the original intervention of the reoperation when repeated surgery was needed remained unchanged until 4 years after the last operation which was the follow up time in all cases.

(1) Biochemical results

When no operation is performed normal tear total protein content (approximately

800 mg/100 ml) is restored about 20 days after an alkali burn. Normal levels of total protein and normal protein fraction composition are restored about 3-4 weeks after the free transplantation of conjunctiva from the other eye. No delay in healing after grafting with buccal mucous membrane and 8 days after implanting Bioplast fibrin film (Tapasztó 1973).

## Discussion

Recording the clinical course, total tear protein, tear protein fraction composition and the cosmetic results showed the Bioplast fibrin graft to be a favorable conjunctival substitute. It is equivalent to autogenous conjunctiva and can be recommended in severe chemical burns highly limiting the availability of healthy tissue. The fibrin implant irritates the eye to the least degree, is replaced by conjunctiva as it acts almost as a template for the proliferation of fresh tissue and neovascularization from the episclera and surrounding healthy conjunctiva. Neovascular conjunctival tissue is found at the site 2-3 weeks postoperatively.

Suppuration is readily prevented by the postoperative treatment. No regression occurs and the cosmetic results are good after several years. The time of postoperative healing is not longer than after free conjunctival transplantation and is shorter than after implanting other materials. The Bioplast implant is transformed into functional conjunctiva.

In traumatic pterygium Bioplast is indicated only if there is a good prospect that the corneal epithelium can be restored before the implant is degraded and new tissue develops.

With older, scarred injuries and symblepharon the implanted Bioplast film can be in contact with the wound surface on one side only. The other side of the implant should lay free or if it is in fornices the conjunctiva must be healthy on the other side of the fornix.

Bioplast was found particularly helpful in older injuries causing the formation of distorting scars, thus reducing the amount of healthy conjunctiva. When the scarred tissue is excised, large areas can be covered with the graft, stimulating the formation of new conjunctiva and yielding good cosmetic results.

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*Sixth Congress of the European Ophthalmological Society*

The sixth meeting of the European Ophthalmological Society will be held in Brighton, England from 21st to 25th April 1980.

The main theme for the scientific programme will be 'The Cornea in Health and Disease'. Subsidiary meetings on other subjects will be held in association with the main meeting and will include round table discussions and workshop sessions.

Papers should be submitted to Professor W. S. Foulds, Department of Ophthalmology, University of Glasgow, Glasgow, Scotland.

Further information can be obtained from the Official Organiser, Holland Ophthalmic Centre, 16 Lange Voorhout, The Hague, The Netherlands.

The centenary meeting of the Ophthalmological Society of the United Kingdom will be held in London during the week preceding that of the European Society meeting in Brighton, 18th April 1980.

Further information for this meeting can be obtained from The Honorary Secretary, Ophthalmological Society of the United Kingdom, The Royal College of Surgeons, Lincoln's Inn Fields, London WC2, England.

*Postgraduate course of the University of California on Glaucoma*

The postgraduate course of the University of California School of Medicine (on Glaucoma) is scheduled to be held at the St. Francis Hotel on Union Square in San Francisco, February 6-8, 1980.

For further information please contact: Extended Programs in Medical Education, Room 369-L, University of California, San Francisco, California 94143 or call (415) 686-4101.

*2nd International Congress on Vision and Road Safety Paris*

is held from 20th to 22th November 1980. Night Driving is the conference theme. Lighting, Vehicle Lights, All night Road Signs and Signals, and Reflectors are main themes. Numerous round table conferences.

Information: R. Pansard, General Secretary, La prevention routiere internationale, 91310 Paris Montlhery, France.

# 24th Nordic Ophthalmological Congress 1979

held in Oslo from 13th to 16th June 1979 with the cornea and ergophthalmology as the main scientific topics. The president was professor Jan Yteborg. The topic of the plenary meeting was organization and resource problems concerning the combined visual and eye care services. The plenary meeting closed with the presentation of the K. A. K. Lundsgaard Medal. The gold medal and prize was to be awarded to the author of the best article in *Acta Ophthalmologica* volume 55 and 56 (1977 and 1978). The candidate was chosen on recommendation by all the Nordic professors as requested by the testamentary articles. According to my opinion, were worthy of a gold medal. The choice of Ole Nissen from the Eye Department, Rigshospitalet, Copenhagen.



Nissen was selected because of his basic study of the disease process which is a threat to us all and which represents a scientific challenge, namely glaucoma. Ole Nissen has developed a completely original method for the constant measurement of the intraocular tension by means of a so-called suction-cup fixation method in studies on rabbits and humans (*Acta ophthalm. (Abh.)* 55: 60).

Mogens Vorn

*From The Department of Ophthalmology (Head N. Ehlers)  
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## CORNEAL TRANSPLANTATION AND HLA HISTOCOMPATIBILITY

A preliminary communication

By

NIELS EHLERS and FLEMMING KISSMEYER-NIELSEN

A series of 222 cases of 7 mm penetrating corneal grafts were analysed with respect to the influence of HLA compatibility. The degree of compatibility was random as no matching was done (HLA types unknown at time of operation). Consequently most of the cases showed 3 or 4 incompatibilities.

The series was divided into seven diagnostic groups (keratoconus, herpetic keratitis, non-herpetic keratitis, stromal dystrophy, endothelial dystrophy, mechanical lesion and corrosion). In all groups there was a tendency towards better results among the compatible transplantation, but only when considering the entire series could statistical significance be demonstrated. The groups of 0-2 incompatibilities showed fewer rejection episodes or opaque grafts than the groups of 3 or 4 incompatibilities ( $\chi^2 = 9.70$ ,  $P < 0.005$ ). Comparing only the frequency of opaque grafts among the two groups the correlation was less significant ( $\chi^2 = 3.66$ ,  $P \sim 0.05$ ).

*Key words:* cornea-graft-tissue typing

### Resumé

The significance of an allograft reaction as the cause of graft failure has, over years, become more and more clear. To day there is no doubt that immune reactions do occur (Grunnet et al. 1976) and the importance of the histocompatibility system, the HLA system, has been demonstrated (Ehlers, Kissmeyer-Nielsen 1973, 1977; Gibbs et al. 1974; Vannas et al. 1976; Sinker et al.

van Alphen 1978) It is probably fair to say that to date no series has been intended to prove the clinical value and show the place of HLA typing in future practice

Since 1968 we have performed 316 corneal transplantations of which 239 were penetrating grafts where in addition the HLA A and B types of donor and recipient were determined Blood samples for typing were drawn after the transplantation and no matching of donor and recipient was attempted The series therefore from a statistical point of view can be considered suitable for an analysis of the influence of HLA compatibility on the fate of the graft

In the analysis of the series 17 cases had to be excluded for the following reasons surgical 6 cases primary graft failure 1 panophthalmia 1 intraocular haemorrhage 1 (undetected haemophilia) severe herpetic reinfection 2 glaucoma 2 and in 1 case antibodies in recipient's serum directed against the donor (acute failure)

The series of the remaining 222 cases is presented in Table I The diagnostic group non herpetic keratitis includes all cases where none of the other diagnoses is appropriate

Considering the results the outcome of the transplantation was described as 1) clear graft no rejection episodes 2) clear graft but definite rejection episodes managed by treatment (Ehlers & Bramsen 1978) or cured spontaneously (2 cases) 3) opaque graft The cases were placed in the three categories by one of the authors (VE) without knowledge of the degree of compatibility between donor and recipient

Generally the results were as could have been expected e.g. good in the groups keratoconus stromal dystrophy and mechanical lesion less favourable in the groups of herpetic keratitis non herpetic keratitis and endothelial dystrophy and very unfavourable in the group of corruptions

*Table I*  
Diagnosis of 222 corneal transplantations

Diagnosis	Women	Men	Total
Keratoconus	13	41	54
Herpetic keratitis	91	22	43
Non herpetic keratitis	34	18	52
Stromal dystrophy	4	11	15
Endothelial dystrophy	20	13	33
Mechanical lesion	9	8	10
Corruption	1	14	15
Total	92	197	222

Table II  
The overall result of 292 corneal transplantations.

	No. of incompatibilities				
	0	1	2	3	4
Clear graft no rejection	2	5	36	59	54
Clear graft + rejection	—	—	1	9	11
Opaque graft	—	—	5	23	14

The overall result of the 222 transplantations (in June 1979) is shown in Table I. It is seen that most transplantations showed 3 or 4 incompatibility. Considering 0, 1 and 2 incompatibilities as rather favourable and 3 or 4 as unfavourable it can be shown by a  $2 \times 2 \chi^2$  table that rejection episodes or opaque grafts occur significantly more frequently among the incompatible transplantations than among the compatible ( $\chi^2 = 9.20$   $P < 0.005$ ). From a more clinical point of view one can distinguish between clear and opaque grafts and it can be found that more opaque grafts occur among the incompatible than among the compatible transplantations. However the significance is less ( $\chi^2 = 3.66$   $P \sim 0.05$ ).

In a following publication a more detailed presentation of our data will be given with calculated actuarial survival curves for the grafts. This has already been done for the first 140 of the cases (Ehlers & Küssmeyer Nielsen 1977). The same analysis confirmed the statements in the literature that the ABO compatibility is of no significance.

The points to be discussed here are the possible consequences to be drawn from the accumulating evidence for the significance of histocompatibility for the result of corneal grafting. Evidently one way is to continue as we have done until now grafting without respect of the compatibility and regraft the inevitable failures. This attitude can by no means be disregarded when e.g. the favourable results obtained in keratoconus are considered. However rejections also occur in keratoconus and the problem increases with no of retransplantations and in other diagnostic groups. A more satisfactory approach would be to attempt to develop an organisational structure giving the possibility of selecting compatible donor-recipient pairs. This could be achieved by having a large pool of HLA typed recipients waiting for the right donor tissue or by having a large pool of HLA typed donor corneas waiting for their recipient. For obvious reasons the latter possibility is preferable and we have therefore developed a freezing technique which will enable us to keep donor tissue at the temperature of liquid nitrogen for prolonged periods. These corneas have now been used successfully in several cases but the

question still to be answered is how the advantage obtained by using compatible compares with the immediate postoperative problems introduced by using rejected tissue.

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## ORGAN CULTURED DONOR MATERIAL FOR PENETRATING CORNEAL GRAFTS

A preliminary report

BY

ALBERT KOLSTAD

Fresh and undamaged cornea had unchanged endothelial cell density for four weeks in culture while eyes removed at autopsy suffered a cell loss. Acceptable cell density was found in eyes removed within eight hours, and this was confirmed by the results of 55 penetrating grafts using cultured autopsied material. Storage in culture for more than one week seemed to influence the results unfavourably. Immune reactions were not seen.

*Key words:* cornea - cornea/transplantation - tissue donors - organ culture

Traditional storage of the donor eye in a moist chamber at 4°C limits its use. This may cause practical problems both for the patient and for the surgeon.

We have therefore looked for methods which will prolong the keeping time of the donor corneae and have found that organ culture fulfills our requirements and brings additional advantages.

The culture of corneal tissue is no recent achievement. Its lack of vascularization makes the cornea undemanding and successful culture of endothelium was first reported in 1928 (Matsui). Cultured donor material for penetrating grafts was suggested in 1948 but was a failure (Hoof 1948, Hoffman 1949).

During the last five years the interest for this approach has revived, and different varieties of the culture method have been published (Summerlin et al 1971, McCarey et al 1974).



have used the method which is common for other mammalian tissues culture enriched with serum and kept at 37°C (Paul 1970)

# Own Investigations

Survival of the endothelium in culture is a crucial question. To answer this we used human eyes recently removed for intraocular malignancy. Four buttons were punched from each cornea and these have been kept in culture for different periods of time before being stained with silver and the cell density determined (Oh 1963).

It shows that cell density in non-traumatized fresh eyes will remain practically unchanged for four weeks in culture.

If the endothelium has been subjected to mechanical trauma, such as bending of the cornea, rows of silver-stained cells will be seen corresponding to the folds in the endothelial membrane (Fig. 2). After a few hours in culture, the undamaged neighbour

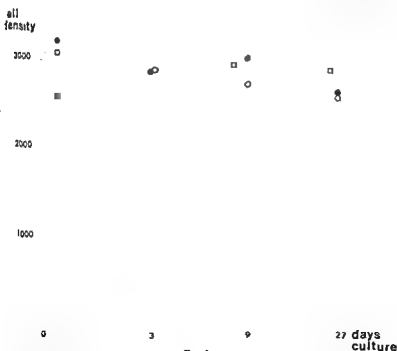


Fig. 1

Endothelial cell survival in fresh undamaged corneal buttons kept in organ culture at 37°C for up to four weeks. Cell density, which is expressed in cells/mm², is determined by counting cells in the microscope after silver staining. Filled circles, open circles and squares denote corneal samples from three separate eyes removed for intraocular tumour.

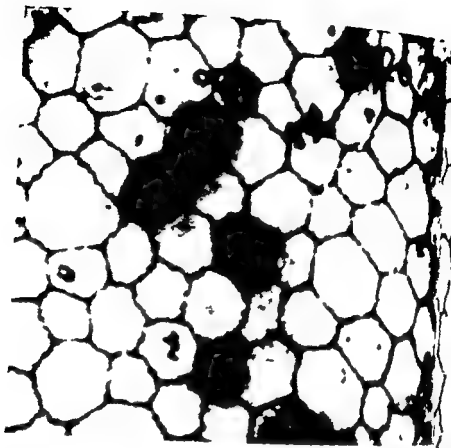


Fig 2

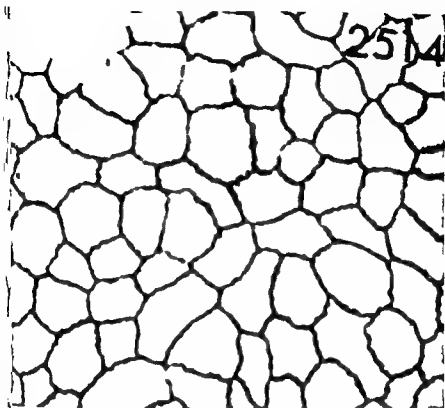
Silver stained endothelium of corneal button. The dark cells are the result of trauma. Cell density 2671 cells  $\text{mm}^2$

cells will expand to fill the space of the dead cells and debris thereby in a regular hexagonal pattern Fig 3

Fig 4 shows the resulting fall in cell density during the first three days. The now remaining are thought to be alive

The supply of fresh cornea from eyes removed for intraocular tumors will answer the demand for donor eyes. We therefore use eyes removed in connection with autopsy

We know that long delay between death and the removal of the eye is not compatible with a viable endothelium. To determine how soon this becomes the case we have removed the eyes at various intervals after death and counted surviving cells after three days in culture. Fig 5 shows the decrease in cell density with increasing post mortem time



*Fig 3*

er stained endothelium of corneal button exposed to mechanical trauma after organ culture for 24 h. The dark cells have disappeared. Cell density 2514 cells/mm<sup>2</sup>

It is difficult to state the minimum cell density which can be accepted for donor material. The mechanical trauma inflicted on the endothelium during surgery could require cell density far above the limit for barrier function which probably is in the neighbourhood of 2-300 cells/mm<sup>2</sup> (Fig 6).

If we choose 2000 cells/mm<sup>2</sup> as the lowest acceptable density for the donor endothelium, Fig 5 shows that this was obtained by removing the eyes before 8 h post mortem.

High donor age will adversely affect cell density (Lang et al 1976). When there has been a choice between eyes with low post mortem time and low donor age, we have preferred the former.

The eyes are removed by a technician using unsterile, clean instruments. It is stored in a sterile vessel in the refrigerator, usually for no more than one h, before being prepared for culture.

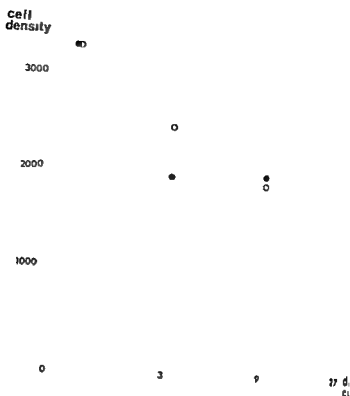


Fig 4  
Endothelial cell survival in fresh corneal buttons exposed to mechanical trauma

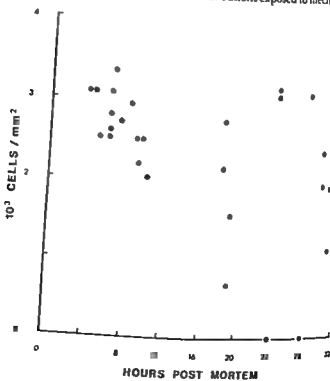


Fig 5  
Endothelial cell survival in eyes removed at various time intervals after death. Cell density determined by silver staining after three days in organ culture

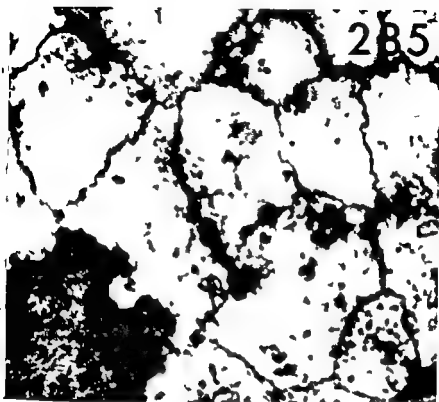


Fig 6

er stained endothelium of corneal button removed from patient with Fuchs dystrophy. In  
te of maximal extension of endothelial cells area of Descemet's membrane is left uncovered  
(lower left) Cell density 985 cells/mm<sup>2</sup>

First of all the epithelium is removed. This will reduce the risk of infection and  
it also prevent the epithelium from migrating onto the stromal section. The  
cor button is then trephined to the correct diameter, the section finished with a  
razor blade fragment and the button transferred to a test tube with 10 ml medium.  
Here it will settle endothelial side up on the bottom of the tube. It is then placed in  
an incubator at 37 C and the medium changed once a week.

We use Eagle Minimum Essential Medium enriched with 10% inactivated human serum  
and with 9 mM l glutamine and 5 mg/100 ml Centamycin added. We prefer the Hepes buffer  
(0.03 M) which will guarantee a correct pH in an open vessel. We can therefore place the  
cor button in a Petri dish on the instrument table before surgery begins.

The donor button is edematous when removed from the medium to be placed in  
the host. Care should therefore be taken to place the sutures deeply close to

Table I  
Results using organ cultured donor material for  
penetrating corneal grafts

Diagnosis	Number	Clouds
Keratoconus	96	0
Bullous keratopathy	16	2 (4)
Maculae corneae	11	1
Dystrophy	5	0
Rejection	2	0
Descemetocoele	1	0
Total	55	3 (4)

hours post mortem

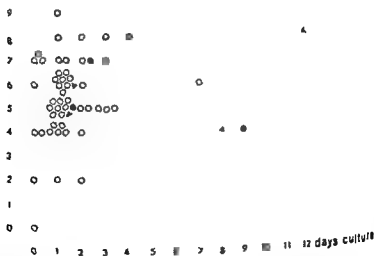


Fig 7

Results of penetrating grafts seen in relation to post mortem time and culture time of donor material. Open circles: clear. Filled circles: cloudy from start. Filled triangles: clear but later than six months. Later gradual clouding.

Descemet's membrane. The donor button will start clearing up during suturing and usually of normal transparency and thickness when pad and bandage is removed two days.

We have not seen microbial growth during culture nor post operative infection. During the last three years we have used cultured donor material for 55 transplanting grafts. Table I shows that three of these were cloudy from the first day of bandage while another four turned gradually edematous after more than six months.

Most of the failures were in patients with bullous keratopathy where the host cornea cannot be expected to contribute with any endothelial cells to the graft.

Figure 7 demonstrates the relationship between post mortem time, culture time and graft failure. Postponing removal of the donor eye up to eight hours after death did not affect the results while storing the button in culture for more than a week tended to increase the number of failures. We have for obvious reasons not tried to expand our material in this direction.

For all practical purposes a limit to storage in culture of one week should be adequate.

We have had no case of immune reaction. One possible explanation is that the donor button discards the dead cells in culture and therefore presents a smaller antigenic stimulus to the host immune system.

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## KERATITIS DENDRITICA

### An epidemiological investigation

BY

K KAMP MORTENSEN and A. K. SJØLIE

One hundred and seven outbreaks of dendritic keratitis have been registered by the ophthalmologists over a 2 year period in a region with a population of approximately 446 000 persons. The incidence was found to be 12.1/100 000 year.

The average age of our sample was 46.5 years with the same age distribution for males and females and with a non significant predominance of males.

No seasonal variation of dendritic keratitis was observed. 50% of the patients had previously suffered from dendritic keratitis. In 95% of the patients the onset of the disease had been preceded by an infectious disease while 67% on local steroid therapy for non-dendritic eye diseases prior to the dendritic outbreak.

**Key words:** keratitis dendritica - herpes simplex virus (HSV) - incidence - seasonal variation - recurrent infection

Herpetic keratitis is a frequent cause of infectious corneal disease in the less developed countries (Howard & Kaufman 1962) and produces with its tendency to recurrence a number of cases of serious visual sequelae. Many experimental and epidemiological investigations have been carried out in order to obtain a better understanding of the frequent recurrences (Nesburn 1975; Baninger & S. 1973; Bastian et al. 1972). HSV infection is a ubiquitous and frequent infection; thus serological investigations have demonstrated that 90% of persons over the age of 20 years have circulating HSV antibodies (Nesburn 1975). Furthermore, approximately 90% of HSV infections are subclinical (Leopold & Sery 1971).



The epidemiological investigations previously carried out have mainly been formed on hospital patients. We have therefore found it of interest to carry out an epidemiological study of dendritic keratitis in a well-defined geographical area.

## Material and Methods

In a pilot study of 3 months duration registration was carried out of all patients coming from dendritic keratitis treated by the ophthalmologists in the county of Funen during the period 1/6 1976 to 31/5 1978. The county of Funen has a population of 446 223 persons (Danmark's Statistik 1977).

Information was obtained of the age, sex, previous infectious diseases, herpes simplex or traumas, and earlier attacks of ipsi- or contralateral herpetic affection. Also those patients who had been on local steroid treatment for an eye disease other than dendritic keratitis were noted.

$\chi^2$  test, F test and  $\gamma^2$  test were employed in the statistical analyses. A significance level of 5% was chosen.

## Results

A total of 107 outbreaks were registered, giving an incidence of 12/100 000/year. One patient suffered three attacks and five patients two attacks of dendritic keratitis during the period of the investigation. The registered patients (45 women, 62 men) were found to have an average of 46.5 years, range 4 to 86 years, as can be seen in Table I. Statistical analysis did not reveal any significant differences in the age distribution between the two sexes, nor in the sex ratio. However, in the county of Funen there are more women than men in the elderly age groups (Danmark's Statistik 1977).

The distribution according to the seasonal outbreak of the disease is shown in Table II, where there was no seasonal variation, neither monthly nor quarterly, as the disease arises evenly throughout the whole year.

Of the three of the 107 outbreaks occurred in patients who had previously suffered from dendritic keratitis in the same eye, while four occurred in patients who had previously had the affection in the contralateral eye, as can be seen in Table I. Only 10 patients had previously suffered from bilateral infection.

Twenty seven patients stated that the corneal affection had been preceded by a common cold, influenza or herpes labialis.

Six patients were being treated with a local steroid for an eye disease other than dendritic keratitis prior to the present outbreak.

Table I

Age and sex distribution as well as the recurrence rate of 10 outbreaks of dendritic keratitis

Age	Females	Females with earlier dendritic keratitis		Males	Males with earlier dendritic keratitis	
		Ipsilat.	Contralat.		Ipsilat.	Contralat.
0-9	1	1	0	4	1	0
10-19	7	2	0	4	0	0
20-29	3	1	0	8	2	1
30-39	5	3	0	10	8	1
40-49	6	1	0	7	4	1
50-59	11	7	0	11	5	0
60-69	7	4	0	11	5	1
70-79	3	2	0	7	3	0
80-89	2	2	0	9	1	0
Total	45	23	0	69	30	3

Table II

The seasonal distribution of 10 outbreaks of dendritic keratitis registered over a period of 9 years

Month	Females	Males
January	4	9
February	3	5
March	3	8
April	4	1
May	4	5
June	8	6
July	1	8
August	6	5
September	6	3
October	4	4
November	1	5
December	1	3
Total	45	69

## Discussion

ous epidemiological investigations regarding dendritic keratitis have mainly based on hospital materials thus Jonkers (1962) collected all the clinical and latent (sic) cases of herpes simplex corneae at the Rotterdam Eye Hospital in 1958, 1959 and 1960 and found a frequency of herpes corneae of 1 out of 100 patients. Norm (1970) found among all the patients referred within a year period to the Department of Ophthalmology Kommunehospitalet in Copenhagen the incidence of herpetic keratitis to be 5.9/100 000/year. Leopold & Sery (1963) as well as Caroleo (1970) have also carried out epidemiological studies of dendritic keratitis based on hospital materials but the incidences are not stated. Thygerson (1976) studied a total population of 800 000 persons and found that the incidence of dendritic keratitis was 4/100 000/year while the incidence in the present investigation is 12/100 000/year as based on the total population of a defined region. Ophthalmological service is readily available within this area and it must therefore be presumed that the majority of patients developing dendritic keratitis consulted an ophthalmologist and thus have been registered. The incidence found in the present investigation will be a minimum figure. Due to the transient symptoms a number of patients may not consult their physician and spontaneous healing is seen in 10–25% (Thygerson 1956, Laibson 1964). Truszkla (1968) found a predominance of patients in the age group 0 to 9 years. Norm (1970) found a predominance in the age groups 0 to 9 and 40 to 60 years while Leopold & Sery (1963) observed the highest incidence between the ages 40 and 60 years. A predominance of men has been seen in all the studies. The present investigation shows no significant difference in the sex distribution between the two sexes even though there is a non significant predominance of men. The distribution of the population within the county of Funen is not similar for both men and women as there are more women than men in the elderly age groups. In the present material there are too few patients in the age group 0 to 9 years in relation to the age distribution of the population within the area. In addition there are relatively too many men over the age of 40 years. A possible explanation of the distribution of fewer children in this material as compared to those of earlier studies may be that children in contrast to adults are more often referred to a hospital due to the difficulty in treating them. For this reason children will comprise a relatively larger percentage of a hospital material than of the registered patients in a survey based on the total population. The large number of middle aged and elderly patients found in the other investigations may be caused by a poorer immune response in the elderly age groups. Seasonal variation has been observed in the outbreak of herpetic keratitis in most of the previous investigations. In particular attention has been given to the

possibility of an increased incidence in the winter months, together with frequent upper respiratory tract infections. The present investigation has demonstrated in good general agreement with Vorn (1970) and Carollo (1962) that there is no seasonal variation. The rate of recurrent attacks is high, 4.7% in that of Jonkers (1962) and 61% in that of Vorn (1970). This investigation has shown that 50% of the patients had ipsilateral recurrence while 4% had contralateral recurrence. The high rate of ipsilateral recurrence supports the theory that recurrence results from an activation of virus elements, which are dormant in the trigeminal ganglion.

The present investigation does not permit any definite conclusions here, and as to the trigger mechanism responsible for the activation of these *in vivo* reflexes.

## Acknowledgments

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PRESENILE CORNEAL ARCUS IN  
HEALTHY PERSONS  
A POSSIBLE CARDIOVASCULAR RISK INDICATOR  
IN YOUNGER ADULTS

BY

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The results of blood lipid screening of 900 39-49 year-old participants with incidentally diagnosed corneal arcus from the Copenhagen City Heart Study are reported

A large sample of study participants of the same age-group without arcus served as controls

In general the arcus group had higher serum-cholesterol than the control-group ( $P < 0.01$  in males and  $< 0.02$  in females). The serum-triglycerides in the two groups did not differ

A trend towards positive correlation between arcus-intensity and cholesterol level was demonstrated by non parametrical statistics. By use of the same statistics an association between lipids and arcus-localisation was searched out. Dense arcus in the nasal lower limbal regions occurred together with hypercholesterolemia more often than did a marked upper outer arcus

Consequently based on the present investigation serum-cholesterol screening of middle aged persons with marked lower nasal arcus seems justified, since hypercholesterolemia might well be an underlying cause

Finally the importance of diagnosing and treating hypercholesterolemia is briefly discussed

*Keywords:* corneal arcus - slit lamp grading - prevalence in middle age - hypercholesterolemia - serum triglycerides - serum glucose - arcus-intensity - arcus localisation

Our present knowledge regarding corneal arcus in relation to lipid abnormalities and cardiovascular disease/death dates from several sources. A correlation between arcus in pre middle age and acute myocardial infarction has been established in a large scale prospective population study, the Western Collaborative Study (Jaeger & Eisenhauer 1977) whereas others have reported a lack of relation between atherosclerosis verified by autopsy and arcus prevalence (Strom 1967).

Screenings for arcus prevalence and arcus-degree have established correlations between hyperlipidemia, especially hypercholesterolemia, and arcus (Jaeger & Eisenhauer 1977). The same investigators further stressed the importance of lower (= inferiorly located) arcus in relation to blood lipid abnormalities.

Reports on lipid screening on normals with incidentally diagnosed corneal arcus are rare and the results are controversial (Parwaresch *et al.* 1976).

To our knowledge no earlier attempts have been done on a larger scale to evaluate the clinical importance of both arcus intensity and prevailing arcus-localization in symptom free individuals.

So the aim of the present study is to analyse the correlations between blood lipids, in this case cholesterol and triglyceride and corneal arcus in a group of presumed healthy pre middle aged persons.

## Material

From the total group of participants in the Copenhagen City Heart Study (abbreviated CCHS) aged 39–49 years in 1977 those persons were selected for study in whom a corneal arcus was macroscopically diagnosed by the participating cardiologists. Before inclusion into the study the persons underwent an ophthalmological examination to verify the arcus and to exclude persons with gross ocular pathology from the study.

As blood parameters the non fasting values of serum-cholesterol and serum-triglyceride and glucose (mmol/l) were chosen.

Out of 270 persons 200 persons (117 males and 83 females) participated in the study and constitute our arcus group.

As blood sample-controls served the total group of participants in the CCHS aged 39–49 years in 1977 (1440 males and 1705 females). They were located in the data registration available; accepted as controls because of the low prevalence in the group, about 6.8%.

## Methods

### *Liminary procedures*

ophthalmic investigations were performed by one of us (L. Varnek) over seven  
his Prior to the slit lamp examinations every person received a letter  
inng why where and when to meet. Eighty five % of those who received the  
attended for examination

### *Examination proper*

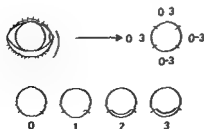
mp examination was performed on right eyes only by the use of a Haag Streit  
mp (type 500) supplied with tungsten light magnification  $\times 15$  and fixed  
diffuse 6 V illumination The corneal arcus was given a total score ranging  
0-12 and a quadrant score ranging from 0-3 in any of the four quadrants  
r lower nasal temporal based on both surface-extension and surface  
ity of the arcus Fig 1 gives examples of this scoring system  
ally the cornea under investigation was photographed with a Zeiss slit lamp  
ra (Ectachrome magnification  $\times 2$ )

### *Scoring methodology remarks*

odological difficulties in obtaining an "exact" arcus scoring were experienced  
both arcus-surface-extension and arcus surface intensity varied along the  
al circumference  
re subjective way of scoring which has been employed by the single observer in  
study was controlled by means of the photographs taken

### *Registration*

person in the study received four registration sheets one for name address etc one for  
trition of known diabetes earlier cerebral strokes or myocardial infarction one for  
train of the blood sample analyses (these three sheets were taken as photocopies from



*Fig 1*

ematic illustration of the employed arcus-scoring system Total score values obtainable  
No arcus = score 0 Weak arcus = score 1 Intermed = score 2 Strong arcus = score 3

the journals on the persons in the CCHS) and finally one for corrected arcus and supplementary corneal remarks when necessary.

Because of the separated sheets the lipid values of the persons in the arcus score were unknown to the slit lamp investigator.

## Results

To assess significance of the observed associations a probability level of  $P < 0.05$  was used.

In the arcus group the mean age of the examined males was 46.3 years (SD 3.1 years) and females 46.6 years (SD 3.1 years).

The two sexes had the same mean total arcus score value of 2.3.

Comparing the whole arcus group with the controls a significantly higher cholesterol level was demonstrated in the arcus group and in both sexes (see Fig. 1a) ( $P < 0.01$  in males and  $< 0.02$  in females).

In the two sexes there were no differences between arcus group and controls concerning triglyceride and glucose levels (see Fig. 2b and c).

Correlations between arcus intensity (score 0-19) and sex analyses were calculated by non parametrical statistical methods (Kruskal Wallis) (see Table I). Especially in females but also in males there was a trend towards a positive correlation between arcus-degree and cholesterol values which nearly reached significance ( $P < 0.074$ ).

Table II shows a significantly higher frequency of high cholesterol values in the female group of high arcus scorers as compared with low arcus scorers ( $P < 0.01$ ) but not in the corresponding male groups ( $P > 0.05$ ).

Finally we wanted to establish whether arcus with shifting localisations (up to 3 temporal nasal) were equally often combined with and thereby the visual expression of an underlying hypercholesterolemia.

The arcus areas selected for investigation were the upper lower nasal and temporal limbal quadrants.

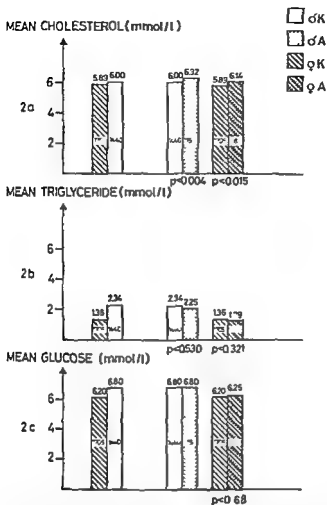
Again by use of the non parametrical Kruskal Wallis analysis (see Fig. 3a) and based on the arcus scoring system from 0-3 in the quadrants earlier determined correlations between increasing score values at the four chosen limbal quadrants and blood sample analyses in either sex were calculated and in total 104  $P$  values resulted.

Out of the 24 possible correlations examined three significant correlations with cholesterol attached were established.

1. Females showed a positive correlation between degree of nasal arcus and cholesterol values ( $P < 0.05$ ).



# Corneal Arcus and Blood Lipids in the Middle-aged



Figs 2 a-c

Bar diagrams showing the mean arithmetic values for non fasting cholesterol (Fig 2 a) triglyceride (Fig 2 b) and glucose (Fig 2 c) in mmol/l in males and females of the arcus and control groups.  $\square$  males control group  $\square$  males arcus group  $\square$  females control group  $\square$  females arcus group. The two first columns (left) show the absence of a sex-difference in cholesterol and the presence of a sex-difference as for triglyceride and glucose. In the last four columns (right) sample means of arcus and control groups are apposed (see text). Numbers within columns show the size of the group under consideration. Numbers at top of the columns show the arithmetic mean value of the relevant parameter. Number at bottom of columns obtained P values in comparing sample means (Student's t test, computer assisted).

Table I

Correlation between arcus intensity and cholesterol, triglyceride and glucose in males and females in the arcus group. A slight positive trend between arcus score and serum cholesterol is demonstrated especially in females. Statistically insignificant, however, at the 5% level (Kruskal Wallis).

Cholesterol		Triglyceride		Glucose	
Males	Females	Males	Females	Males	Females
0.128	0.074	0.780	0.160	0.431	0.25

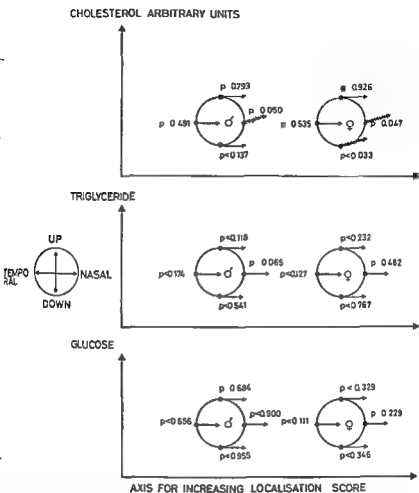
Correlation between serum analysis and arcus intensity *P* values

Table II

Absolute and relative occurrence of serum cholesterol values higher than 7.0 mmol/l (left) and higher than 8.0 mmol/l (right) in males and females of the arcus group further divided into high and low total arcus scores. The relative frequency of high cholesterol values in the "high arcus score group" is impressive. Significance levels ( $\chi^2$  tests) between low and high arcus scorers are shown in brackets.

	No of persons	Persons with se-cholesterol > 7.0 mmol/l		Persons with se-cholesterol > 8.0 mmol/l	
		No	%	No	%
<b>Males</b>					
Low arcus score					
0-5	58	9	15.5	2	3.5
High arcus score			(0.1 > P > 0.02)		(P > 0.1)
6-12	57	20	35.1	6	10.5
<b>Females</b>					
Low arcus score					
0-5	22	3	13.6	1	4.5
High arcus score			(0.01 > P > 0.001)		(0.02 > P > 0.001)
6-12	30	11	36.7	4	13.3

# Corneal Arcus and Blood Lipids in the Middle aged



Figs 3 a-c

non-parametrically calculated correlations (Kruskal Wallis analysis computer assisted) in males and females between relevant serum values and increasing arcus score in the four quadrants. The 6 circles symbolize limbal rings of right eyes. The P value for any given relation (sex/se analysis/arcuslocalisation) is placed in relation to an arrow originating in the localisation under study which for illustrative purposes depicts correlation ( $P \leq 0.05$ ) if it is ascending and zigzagged and lack of correlation ( $P > 0.05$ ) if it runs horizontal and is straight.

Table III

Occurrence of absolute and relative se-cholesterol values higher than 7.0 mmol/l in males of the arcus group, divided into high and low nasal arcus scorers. Significance level ( $\chi^2$ -test) between low and high nasal arcus scorers is shown in the bracket.

Males	No of persons	Persons with se-cholesterol > 7.0 mmol/l	
		No	%
Low nasal arcus score 0-1	87	16	18.4
High nasal arcus score 2-3	27	19	70.4
(0.01 < P < 0.05)			

Table IV

Occurrence of absolute and relative se-cholesterol values higher than 7.0 mmol/l in females of the arcus group, divided into high and low nasal arcus scorers. Significance level ( $\chi^2$ -test) between low and high nasal arcus scorers is shown in the bracket.

Females	No of persons	Persons with se-cholesterol > 7.0 mmol/l	
		No	%
Low nasal arcus score 0-1	62	5	8.1
High nasal arcus score 2-3	22	8	36.4
(0.01 < P < 0.05)			

Table V

Occurrence of absolute and relative *se*-cholesterol values higher than 7 mmol/l in females of the arcus group divided into high and low inferior (lower) arcus scorers. Significance level ( $\chi$  test) between low and high inferior arcus-scorers is shown in the bracket.

Females	No of persons	Persons with <i>se</i> -cholesterol > 7.0 mmol/l	
		No	%
Low inferior arcus score 0-1	37	2	5.4
High inferior arcus score 2-3	47	11	23.4

(0.02 <  $P$  < 0.05)

Males showed a positive correlation between degree of *nasal* arcus and cholesterol levels ( $P = 0.05$ ).

Females also showed a positive correlation between degree of *inferiorly* situated III and cholesterol values ( $P < 0.04$ ).

Tables III, IV and V give support to the three significant correlations.

The other 21  $P$  values, none of which were significant at a 0.05 level, are shown in Tables 3a-c. The uniformly high  $P$  values (cholesterol attached) in relation to *upper* arcus formation are remarkable.

## Discussion

Most authors agree upon the existence of a correlation between arcus and hypercholesterolemia in younger age groups; this association, however, disappears in the older age groups where *local* corneal factors are thought to dominate in arcus formation (Cogan 1974; Immich et al 1967; Parwaresch et al 1976; Jensenmann et al 1974).

Regarding the intermediate age groups, opinions are diverging: one group of authors claiming lack of significance (Immich et al 1967), another an obligate existence of underlying lipid abnormalities (Parwaresch et al 1976). The latter view, however, cannot be true, since the prevalence of lipid abnormalities in the

total population (Wood et al 1972) does not at all reach that of arcus in the age group under study (39-49 years) (10-25% Forsius 1954 Jaeger & Eisenhauer 1977)

As concerns our own results the higher mean cholesterol levels in the female arcus groups are in agreement with results obtained by others (Cogan & Imrich et al 1967 Jaeger & Eisenhauer 1977 Rosenmann et al 1974). While the total occurrence of hypercholesterolemia (serum-cholesterol higher than 200 mg/dl) in the arcus group is 20% (males 2.5% females 15%) (Table II) the overall occurrence of hypercholesterolemia (Fredericksson II) in the general population is only 3.7% (Wood et al 1972)

Our correlation between arcus intensity and serum-cholesterol, demonstrated in women only, has gained less support in the literature. Forsius (1954) correlated increasing arcus intensity in both sexes to increasing blood cholesterol levels only in his younger arcus groups. In contrast Parwaresch et al (1976) have mentioned a lack of correlation between arcus-degree and blood lipids.

Regarding the significance of arcus-localisation we found in accordance with suggestions in the literature (Forsius 1954 Jaeger & Eisenhauer 1977) a positive correlation between dense lower arcus formation in females but not in males, and high cholesterol values.

We also think that the obvious lack of correlation between dense upper arcus and blood cholesterol levels deserves to be mentioned.

Our most significant finding, the correlation between marked arcus formation nasally in both sexes and high cholesterol values, seems to be entirely new. To our knowledge at least a distinction in importance between nasally situated arcus and arcus situated elsewhere has not previously been put forward.

Since several limbal affections preferentially develop nasally, e.g. pingueculae and spheroid degeneration possibly because of local degenerative factors in this region, degenerative corneal changes might be responsible for the above associations. However, a lack of underlying degenerative corneal changes in arcus formation speaks against this (Cogan & Kuwabara 1959).

Summing up, marked arcus formation in both sexes but especially in females and marked nasal arcus in both sexes should arouse suspicion of underlying hypercholesterolemia at least within the age group studied (39-49 years).

Since familiar hypercholesterolemia is associated with serious cardiovascular complications if untreated (Research Committee 1971 Coronary Project 1971) it should deserve early recognition.

The cholesterol lowering effect of diet restrictions and lowering of caloric intake is well established (Parwaresch et al 1976) but during the last few years (Lipson 1978) treatment with lipid lowering agents, especially atomidol and nicotinic acid, has been considered safe and free from disturbing side effects.

used of stated connexion with gall-stones (atromidin) and skin disorders (n) and suspected connexions with gastro-intestinal malignancies precipitation of cardiac arrhythmias and sudden death. Although the clinical benefice of diagnosing hypercholesterolemia in an other symptom free person thus seems debatable better and safer treatment might be available for future use and until then the above mentioned lipid lowering agents are still in widespread use. It would therefore as a prophylactic precaution against hypercholesterolemia best lipid screening on any pre middle aged person with marked especially if marked corneal arcus.

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**Table I**  
**Thickness of the precorneal fatty layer in normal eyes assessed in**  
**interference study Sex and age incidence**

Age	Average $\pm$ SEM (nm)	$\geq 200$ nm (%)	No. of eyes
10-20	133 $\pm$ 90	18	11
20-30	93 $\pm$ 10	0	19
30-40	123 $\pm$ 16	10	10
40-50	97 $\pm$ 13	0	10
50-60	106 $\pm$ 11	9	20
60-70	100 $\pm$ 8	9	43
70-80	93 $\pm$ 8	5	39
80-90	104 $\pm$ 10	4	6
Total	102 $\pm$ 3	53	106
Females	100 $\pm$ 4	55	18
Males	103 $\pm$ 6	51	8

precorneal film and in the presence of a sustably thick fatty layer the interference phenomenon itself can be seen curved and winding brilliantly coloured lines across and beyond the illuminated area

Red interference colours indicate a not less than 200 nm thick fatty layer as waves extinguished being displaced half the wave breadth Blue =  $4000 \text{ \AA}$  to  $2000 \text{ \AA} = 0.2 \mu\text{m} = 200 \text{ nm}$

If red interference colour is not directly visible the examiner must place a mirror on the outside of the patient's lower lid and move this upwards at a slow rate until the red interference phenomenon occurs. If the normal palpebral fissure is reckoned to be 12 mm high and the red colour does not appear until the fissure has been narrowed to 8 mm for instance the fatty layer is estimated to be half of the stated 200 nm i.e. 100 nm thick. The area of the mirror reflected on the cornea is 8 mm high and reaches the centre of the cornea by the fixation employed.

The principle of the semiquantitative method is in other words that of narrowing the palpebral fissure until red interference colour appears. The thickness of the fatty layer (Table I) is calculated on this basis. In patients with presbyopia measurement takes place after distension of the palpebral fissure to a breadth of 12 mm

In rare cases with a very thick fatty layer this is seen as a cloudy white layer with no interference colours (Mc Donald's crumpling phenomenon) (Fig. 2)



he fatty layer is thin weak blue interference lines may be seen prior to  
ence of the characteristic red lines on further narrowing of the palpebral

Donald (1969) used a Goos lamp placed behind the slit lamp for illumination of the  
but undertook no quantification. The Goos lamp has the advantage of allowing the  
cornea to be reflected successively. However in the absence of such an arrangement  
one mentioned ground glass plate is a very useful aid.  
Experiments on oneself are carried out using the concave mirror of a microscope with a + 6  
eyepiece lens attached - mounted on an X ray projector screen according to McDonald or  
from the bulb of a lit Goos lamp.

Statistical method employed is that of Student's *t*-test. The results are expressed as  
 $\bar{x} \pm \text{SEM}$  (standard error of mean).  $P < 0.05$  is considered significant.

## Material

Series of patients studied come from an eye department, an ophthalmic  
surgical department and the author's own ophthalmic practice. The normal  
series comprises untreated patients with no conjunctival complaints referred for  
examination. A total of 206 normal eyes from 166 subjects were examined.  
Age incidence is shown in Table I. The total series comprises 303 subjects with  
either 354 pathological eyes. The diagnoses are shown in Table II.

## Results

### Normal series

Table I shows that in normal eyes the fatty layer has an average thickness of  $102 \pm$   
nm (about 0.1  $\mu\text{m}$ ). In no more than 5 per cent was the fatty layer so thick that red  
interference was visible at wide-open palpebral fissure ( $\geq 200$  nm). The normal  
thickness was evenly distributed corresponding approximately to a gaussian bell  
shaped curve (Fig. 2). No sex difference was noticed. The amount of fat was  
independent of age; an observed tendency towards an increased amount in  
older patients was not significant (Table I).

Twenty duplicate determinations gave a coefficient of variation of 12.7 per cent.

### Pathological series

As might be expected, an increased amount of fat was observed in patients  
suffering from chronic squamous blepharitis with greasy marginal scales. The  
thickness of the fatty layer averaged 129 nm. Maximum thickness was seen in 22 per  
cent (Table II).

Table II

Thickness of the precorneal fatty layer in 504 pathological eyes compared with normal (cf Table I). The last column but one indicates in per cent the number of eyes with maximum fatty layer with red interference phenomenon with slit lamp.

Diagnosis	Average $\pm$ SEM (nm)	P	$\geq 70$ nm (%)	Ref.
Chronic squamous blepharitis	129 $\pm$ 8	<0.001	71	1
Contact lens hard	187 $\pm$ 8	<0.001	8	2
Contact lens soft	147 $\pm$ 18	<0.001	40	1
Cat postop 4-6 days oil	197 $\pm$ 2	<0.001	94	2
Acute infectious conj	193 $\pm$ 3	<0.001	83	1
Chronic infectious conj	164 $\pm$ 7	<0.001	41	2
Obs for infect conj	169 $\pm$ 14	<0.01	51	1
Chronic simple conj	124 $\pm$ 13	n.s.	21	1
Keratoconjunctivitis sicca	163 $\pm$ 9	<0.001	40	2
Recurrent corneal erosion	18 $\pm$ 13	<0.001	0	1
Corneal transplant >4 months	108 $\pm$ 11	n.s.	0	1
Corneal disorders other	137 $\pm$ 19	n.s.	11	1
Iritis	200 $\pm$ 0		100	1
Episcleritis	106 $\pm$ 10	n.s.	21	1
Allergy (conjunctiva)	93 $\pm$ 14	n.s.	0	
Sundry	161 $\pm$ 7	<0.001	33	1

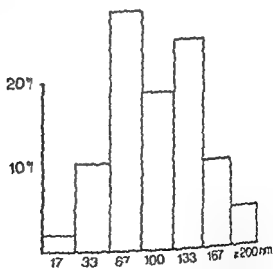


Fig 2

Thickness of precorneal fatty layer estimated by interference study in 504 normal eyes. Mean 102 nm. Abscissa: Thickness of fatty layer. Ordinate: Number of eyes of each layer thickness concerned expressed in per cent.

wearing of contact lenses whether hard or soft, was in most cases associated with increased amounts of fat both across the lens itself (pre-contact lens film) and beneath the lens and also after removal of the lens. The contact lenses looked clean in only a few cases only. In these the fatty layer was as in normal eyes (in 11 per cent of hard lens wearers and in 27 per cent of soft lens wearers).

The amount of fat was even more frequently increased in patients with acute bacterial infectious conjunctivitis and panophthalmia. The method can be employed to distinguish between bacterial conjunctivitis and a sterile conjunctivitis (obsessive conjunctivitis in Table II).

A thick fatty layer was seen a few days after cataract extraction. This may be due either to the postoperative inflammation or to the oil treatment given (ultralanum chloramphenicol 5 mg fluocortolone pivalate and 2 mg chloramphenicol in 0.5 ml oil to 1 g).

In keratoconjunctivitis sicca the amount of fat was increased in the cases with marked clinical signs of bacterial infection. The same was true for patients with keratitis, pharyngeal conjunctivitis, marginal keratitis, exophthalmos, facial nerve palsy and ectropion if concurrent signs of bacterial infection were present.

In patients with chronic conjunctival complaints (simple conjunctivitis) on the other hand there was a normal fatty layer. The same was true for patients with allergic conjunctivitis, epiphora, ptosis, episcleritis, corneal disorders with no signs of bacterial infection (dendritic keratitis, corneal opacity, keratoconus) several weeks

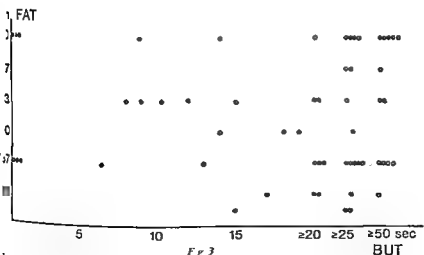


Fig. 3. Relation between stability of the precorneal film and thickness of the fatty layer (73 eyes). Dot diagram. Abscissa: Break up time in seconds. Ordinate: Thickness of fatty layer of precorneal film in nm.

Table III

Thickness of the precorneal fatty layer. Experimental studies. Influence of (liquid paraffin 20% - petroleum jelly 80% mixture) before and after squeezing of a small number of meibomian glands. ultracortinol drops (acetate 0.5% paraffin suspension) silicone oil drops (dimethicon 7 and octadecan 1) polyethylene glycol Lab Kayserberg France) 8) Immediately after 20 min later. A total of 84 experiments.

	Before average $\pm$ SEM	After average $\pm$ SEM	P	Notes
Ointment	76 $\pm$ 8	200 $\pm$ 0	<0.001	1
Squeezing of meibomian glands	57 $\pm$ 7	200 $\pm$ 0	<0.001	
Cocaine 2%	90 $\pm$ 10	113 $\pm$ 19	n.s.	
Ultracortinol drops	123 $\pm$ 23	120 $\pm$ 91	n.s.	
Silicone oil	142 $\pm$ 12	1 $\pm$ 1	<0.001	3
Awakening	200 $\pm$ 0	150 $\pm$ 10	<0.001	2

postoperatively (cataract corneal transplantation). The small number of cases showed increased amounts of fat.

A reduced thickness of the fatty layer was noticed in one group only, cases with a diagnosis of recurrent corneal erosion.

The stability of the precorneal film might be concerned to depend on thickness of the fatty layer. Fig. 3 shows however that examination of 13 out of 39 patients revealed no correlation between break up time and thickness of the fatty layer.

#### Experimental studies

Application of ointment gave a maximum fatty layer on the precorneal film as the ointment had melted and been distributed over the cornea. The lumps of ointment in lumps effected no interference. But after some blinks the ointment distributed in an even layer giving maximum interference (Table III). The thickness of the fatty layer could be seen upto 24 h after the application. For squeezing of a small number of meibomian glands the secretion formed a maximally thick precorneal fatty layer.

The following factors seemed not to alter the fatty layer of the precorneal film: 2% cocaine eye drops, ultracortinol acetate suspension (0.5% paraffin suspension), methylcellulose (mucomimetic), intense rubbing of the eyelids with the finger.

The fatty layer could be reduced by instillation of silicone oil, a water repelling compound reducing the aqueous phase of the precorneal film.

... (1977) The interference phenomenon disappeared completely immediately after instillation. Five to ten min later fairly small islands occurred with red lines which gradually spread with the tear fluid across the cornea. Experiments on myself the amount of fat was found to be at a maximum the next time the eyes were opened in the morning after sleep (Table III). In seven out of ten observations the precorneal film was covered by a silky granular layer giving no interference phenomenon as a thick fatty layer. The results of the investigation bore out this view; maximum interference phenomenon in these cases occurred after few minutes or directly by ectropionising the eye. In the remaining 15 cases maximum interference phenomenon was observed immediately. The fatty layer returned to normal between 20 and 60 min later and remained normal the rest of the day. Staining with alcian blue showed that mucus gives no interference phenomenon and that with fluorescein dry spots on the cornea likewise cause no interference phenomenon. Interference phenomena on the conjunctiva, on contact lenses and on eye prostheses can yield distinct interference phenomena.

## Discussion

Donaldson (1969) characterized the fatty layer on the cornea as a layer proceeding from the lid margins with a drape-effect. In my judgment the fatty layer and the interference phenomenon diffusely cover the precorneal film. By narrowing of the palpebral fissure the phenomenon is recognizable all over, not only along the lid margins but also across the centre. This observation led to development of the method first described in this paper for semiquantitative measurement of the thickness of the fatty layer. According to clinical experience the method is useful for assessing fat induced changes in wearers of contact lenses, in applicants for contact lenses and in patients with blepharoconjunctivitis. Bacterial conjunctivitis is always associated with presence of an increased amount of fat. This is not directly due to the neutrophilic granulocytes which are not available. In the mucous thread in the inferior fornix the fat gathers to form vacuoles while the inflammatory cells are found scattered in the mucous thread (Norm 1974). An increased amount of fat may thus either be due to a primary increased fat production (blepharitis, contact lens) or be secondary to bacterial conjunctivitis. The latter can be demonstrated with greater certainty by a quantitative cytological

conjunctival tear fluid examination or by vital staining with tetrazolium blue (Norn 1974)

In the present investigation observations were made suggesting that a amount of fat on the precorneal film may cause recurrent corneal erosion.

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## QUANTITATIVE MEASUREMENTS OF THE FLUORESCENCE IN LIMBAL VESSELS BY DYNAMIC TELEVISION ANGIOGRAPHY

BY

ERIK SCHERFIG JENS EDMUND and  
HENRIK LUND ANDERSEN

A method for dynamic angiography and quantitative measurement of fluorescence in ocular vessels in an *in vivo* experiment and in corneo-conjunctival angiography is described.

**Key words:** fluorescein angiography - dynamic angiography - fluorescence measurement - television angiography

television exposure is at a rate of 50 images a second and fluorescein angiography performed with TV equipment may be recorded on a tape for later measurement.

In angiography the intensity of the light emitted from the fluorescein depends on the amount of fluorescein in the vessels. This means that measurement of the emission from the vessels at different times of the angiography affords a qualitative expression of differences in the time at onset of vascular filling and an expression of the magnitude of oozing.

## Method

For the television recording of fluorescein angiography we have used a black-white camera having the following specifications:

<i>Type of camera</i>	<i>Sensitivity</i> 1 lux
2/3 inch Chalmicon electrostatic focusing electromagnetic distortion	<i>Automatic sensitivity control</i> by the aid of an automatic lens
<i>Scanning system</i> 625 lines 25 exposures/sec 2:1 interface	<i>Video output</i> 1 volt sync. negative 100 $\Omega$
<i>Horizontal frequency</i> 15 625 kHz	<i>Signal to noise ratio</i> Better than 44
<i>Vertical frequency</i> 50 Hz	<i>Lenses</i> Standard 16 mm C-mount
<i>Linear distortion</i> 1% at 100% utilization of the camera tube	<i>Power requirement</i> 100-197 $\pm$ 0.040 V, 50 Hz, 1 A
<i>Geometric distortion</i> 2%	<i>Power consumption</i> 14 W
<i>Zoom</i> 100-70-50-40% of the total camera tube	
<i>Resolution capability</i> 450 lines	

In dynamic angiography of the anterior chamber the television camera is connected to one ocular of a slit lamp. Through the other ocular the examination is made on the areas to be investigated. Ten ml 5% sodium fluorescein is injected intravenously. The light intensity in the slit lamp was not altered.

Simultaneously with the TV angiography the numbering of each TV image was recorded.

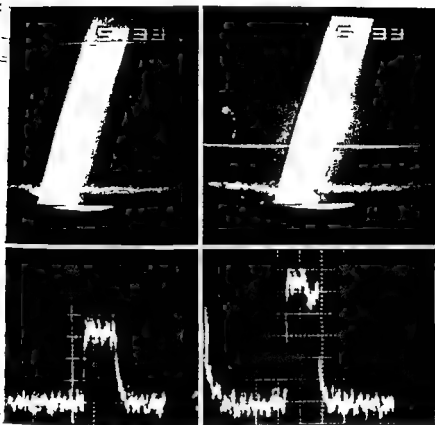
### Measuring method

*In vitro investigation.* A plastic tube is filled with a solution of fluorescein and placed in front of the slit lamp. The television exposure is started and pure water is added anteriorly into the tube. This causes dilution of the fluorescein manifesting a decrease in light intensity on the TV image and a corresponding decrease in the voltage of the video signal recorded on the video tape. The rate at which the decrease in voltage takes place expresses the flow of the injected water.

On each TV image whose number has been recorded simultaneously with the exposure, two points are selected: at a distance of 10 mm on the plastic tube 1) and 2). The voltage of the video signal in the line where the points are selected on the TV image is demonstrated on an oscilloscope (Figs 3 and 4) and the voltage is measured as the height of the excursion on the curve. The width of the pulse is seen as the width of the voltage increase on the oscilloscope curve.

By measuring on image by image a diagram is plotted (Fig. 5) on which the curve represents the change in fluorescence at one point and the other curve





Figs 1-4

- 1 Still of TV image No 333 with recording of the line at which the light intensity is to be measured.
- 2 Still of TV image No 333 with recording of the other line at which the light intensity is to be measured
- 3 Recording on the oscilloscope of the voltage of the video signal through the line in the image in Fig 1
- 4 Recording on the oscilloscope of the voltage of the video signal through the line on image in Fig 2

change in fluorescence at the other point. On the diagram in Fig 3 the decrease in fluorescence - measured as the fall in the voltage of the video signal - is parallel for the two points but chronologically shifted. The chronological shift is 0.08 sec, corresponding to four images. At the selected distance of 10 mm between the points the linear rate is 12.5 cm/sec. At the diameter of plastic tubing used the volume flow is about 0.5 ml/sec.

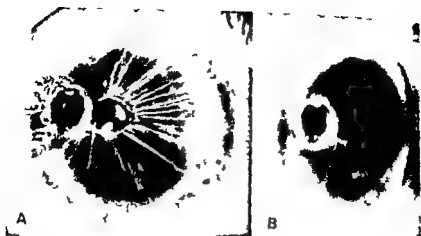


Fig 3

Corneal angiogram in descemetocoele showing (A) small regressive pannus and around descemetocoele blood vessels with (B) diffuse leukemic flow



Fig 4

Angiogram in chronic corneal ulcer showing (A) progressive pannus in all parts of cornea all around limbus and (B) in late phase negative shadow of the cornea surrounding fluorescence corneal ulcer stained with fluorescein (arrow) and the fluorescence of iris

### *Angiography in Corneal Inflammation*

The iris vessels showed fluorescein leakage. The corneal ulcer was not seen in early rein angiograms but in the late phase it showed intensive fluorescence (Fig 1B). After the ulcer had totally healed the corneal macula did not show any marked change in the late phase.

#### *marginal ulcer*

A 45-year-old male had a simple marginal catarrhal ulcer nasally in the left eye. In the rein angiograms adjacent to the ulcer congested conjunctival vessels and dilated capillaries were seen as an early microscopic pannus showing fluorescein leakage and appearance of negative shadows in the late phase. Fluorescein leaked from the limbal vessels into the ulcer which showed increasing fluorescence in the late phase.

#### *Descemetocoele*

A 60-year-old woman was admitted with descemetocoele, small fistulous perforation and iris synechia of the iris in the right eye which had been treated with local antibiotics. In



*Fig 5*

Angiogram in corneal leucoma with lipid keratopathy showing pronounced fluorescence in area of lipid deposition.

fluorescein angiograms the conjunctival and iris arteries filled with fluorescein. A small regressive pannus all around the limbus (Fig 3A). Blood vessels in the late phase surrounded the descemetocoele (Fig 3A,B).

### Chronic corneal ulcer

A 65 year-old male had chronic corneal ulcer in the left eye. Fluorescein angiogram showed progressive pannus invading the peripheral cornea around the corneal ulcer. In the late phase fluorescein leakage from the new vessels which appeared as negative staining surrounding fluorescence in the late phase (Fig 4B). The iris vessels showed early leakage. The corneal ulcer stained with fluorescein in the late phase.

### Corneal leucoma with lipid keratopathy

A 84 year old woman with a history of a corneal ulcer over 10 years earlier had a vascularized leucoma showing lipid deposition in its superior part in the left eye. Fluorescein angiograms narrow radial straight arteries were seen to extend inside the cornea where they formed a capillary plexus. This showed early mottled fluorescence.

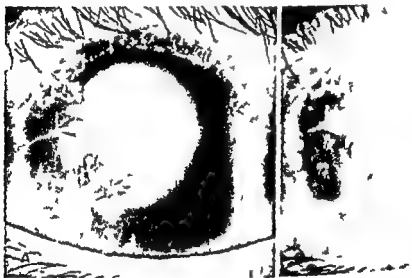
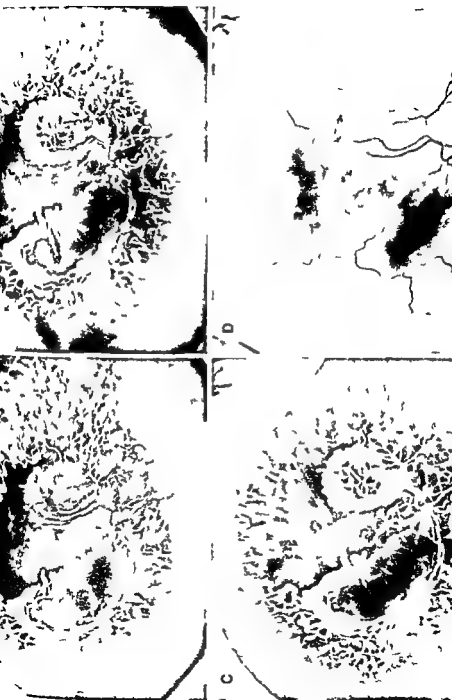


Fig 6

Angiogram in disciform keratitis showing (A) filling of limbal vessels peripheral and early leakage from apex of the vessels and (B) in venous phase peripheral leakage from corneal vessels and peripupillary leakage in the iris.

Fig 7

Corneal angiogram in rosacea keratitis. (A) Filling of limbal pannus area and (B) filling of vascularized pannus in central periphery. (C) Venous phase with profuse fluorescence in the area of the pannus. (D) Late phase with late leakage from the vessels as negative staining surrounding diffuse fluorescence.



which was more pronounced in the area of the lipid deposition (Fig. 5). There was sluggish filling and were seen as negative shadows in surrounding fluorescence phase.

### Disciform keratitis

A 34 year-old male had disciform keratitis in the left eye. Fluorescein angiogram showed progressive pannus with some small vessels loops invading the cornea and the limbus and at the site of the discoid opacity a deep brushlike bundle and two deep bundles with narrow and straight supplying arteries and arborized leakage. Leakage was seen from the apex of the new vessels (Fig. 6A). The new vessels fluorescein leakage (Fig. 6B). In the late phase the corneal vessels were seen as shadows in surrounding fluorescence.

A 28 year-old female had disciform herpes simplex keratitis inferiorly in the cornea. Fluorescein angiograms showed two narrow and straight, deep brushlike branching invading the cornea at 3 o'clock. These deep vessels filled at the same time as the iris vessels whereas a bundle of superficial arborized blood vessels in the limbal plexus at 4 o'clock filled later. Both bundles showed diffuse leakage from the vessels and the discoid oedematous keratitis area stained with fluorescein in phase.

### Rosacea keratitis

A 60 year-old female had rosacea keratitis in the mid peripheral areas of both corneas. Anterior segment fluorescein angiograms of the left eye showed a progressive dilated vessels invading the cornea all round the limbus. Arborescent branching at the end of some vessels. Fluorescein leakage was most conspicuous at the apex of the vessels. Some draining veins showed a circular course. In the late phase of fluorescence the largest vessels as negative shadows was seen in the pannus area.



Simultaneous bilateral anterior segment angiogram in bilateral disciform keratitis and pannus in inferior cornea with discrete leakage and peripupillary leakage of fluorescein (A) and left (B) eye.

9-year-old male had bilateral rosacea keratitis with advanced changes in the right eye. Fluorescein angiograms of the right eye showed progressive pannus with bending vessels extending into the cornea all round the limbus (Fig 7A). A network of vascular in the corneal periphery and a heavy plexus in the scar areas with early fluorescein leakage from the apex of the vessel loops were observed (Fig 7B). In the venous phase the areas showed pronounced fluorescence (Fig 7C). In the late phase the coarse vessels were seen as negative shadows in surrounding diffuse fluorescence (Fig 7D).

#### Keratoconjunctivitis

40-year-old woman had sclerokeratoconjunctivitis in both eyes with sluggish conjunctival action, corneal involvement, anterior uveitis and in the right eye vitritis and cystic lamellar oedema. Simultaneous bilateral fluorescein angiograms of the anterior eyes showed pannus in the inferior perilimbal areas with discrete leakage from the tip of the vessels and pupillary leakage of the iris vessels (Fig 8).

#### Keratoconjunctivitis in rheumatoid arthritis

40-year-old woman with seropositive rheumatoid arthritis, keratoconjunctivitis sicca and a history of a cataract extraction in the right eye eight years previously was admitted with a pannus formation in the perilimbal area of the right cornea superiorly. In fluorescein angiograms the adjacent conjunctiva showed narrow arteries and congestion of the conjunctival capillaries and veins. A superficial pannus extended into the gutter as tortuous vessels with discrete leakage.

#### Mooren's ulcer

After cataract extraction a 63-year-old male developed Mooren's ulcer which spread from operation scar over the whole cornea in 1 year and 9 months. Fluorescein angiograms 10 months after cataract extraction showed narrow and straight conjunctival and thickening episcleral arteries supplying the ulcer area which was invaded by congested limbal



Fig 9

Angiogram in Mooren's ulcer. (A) Congested limbal arcades with early leakage in ulcer area. (B) Diffuse leakage from limbal arcades and from iris vessels.

arcades (Fig 9A). These arcades, as well as the iris vessels, showed early peripheric limbic veins showed marked congestion with sluggish filling (Fig 9B). In the ulcer area and the iris showed diffuse fluorescence.

### Corneal graft vascularization

A 36 year-old male developed leucoma in the left cornea following a trauma. Ten years after the accident a penetrating graft was performed through the graft. Angiogram 20 days after the penetrating graft showed a vascular junction into the lamellar graft and a few deep vessels extending to the penetrating junction where they showed a circular course with some vessel leakage in the penetrating graft. Diffuse leakage was seen in the penetrating graft interface and at the vessels in the graft itself.

A 29 year-old male was grafted due to vascularized leucoma following a trauma the right eye two years earlier. He developed vascularized graft rejection, twice with 700 rad  $\beta$  irradiation. Angiograms five months after corneal transplant showed heavy congestion of conjunctival and perilimbic vessels and extension of vessels into the periphery of the graft with profuse leakage. The iris vessels showed fluorescein leakage (Fig 10).

### Complicated acute keratoconus

A 23 year old male with Down's syndrome developed acute keratoconus in the left eye was complicated by staphylococcus aureus ulcer. Angiograms one month after the disease showed congestion of the conjunctival and limbic vessels with extensive progressive pannus into the cornea all around the limbus (Fig 11A). The periphery showed fluorescein leakage from the apex of the vessels which were seen as neovascularization surrounding fluorescence in the late phase (Fig 11B). The corneal ulcer healed. Oedema decreased during vascularization of the cornea.



Fig 10

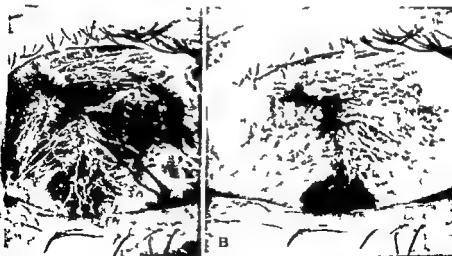
Angiogram of eye grafted for leucoma following tear gas injury shows extension of vessels into periphery of graft (B) profuse leakage from new formed vessels and leakage in iris.





*Fig 11*

cornea in acute keratoconus with staphylococcus aureus ulcer (A) Extension of limbal vessels into the cornea with leakage at the apex of the vessels (B) Late phase with peripheral vessels as negative shadows in surrounding fluorescence and accumulation of fluorescein in margins of central oedematous cornea



*Fig 12*

angiogram in multiple pterygia showing (A) filling of straight arteries and branching vessel spaces and (B) in venous phase extensive network of capillaries and draining veins with discrete leakage

arcades (Fig 9A) These arcades as well as the iris vessels showed early peroxide staining. The limbal veins showed marked congestion with sluggish filling (Fig 9B) Late leakage from the ulcer area and the iris showed diffuse fluorescence

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A 36 year old male developed leucoma in the left cornea following a calow. Ten years after the accident a penetrating graft was performed through the graft. Angiogram 20 days after the penetrating graft showed a vascular network into the lamellar graft and a few deep vessels extending to the periphery where they showed a circular course with some vessel leakage. Diffuse leakage was seen in the penetrating graft interface and the vessels in the graft itself

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### Complicated acute keratoconus

A 23 year-old male with Down's syndrome developed acute keratoconus in the right eye which was complicated by staphylococcus aureus ulcer. Angiograms one month after the ulcer disease showed congestion of the conjunctival and limbal vessels with extensive leakage of fluorescein into the periphery of the cornea all around the limbus (Fig 11A). The periphery of the cornea showed fluorescein leakage from the apex of the vessels which were seen as a late phase of leakage surrounding fluorescence in the late phase (Fig 11B). The corneal ulcer healed and the edema decreased during vascularization of the cornea



Fig 10

Angiogram of eye grafted for leucoma following tear gas injury shows extension of vessels into periphery of graft (A) profuse leakage from new formed vessels and leakage from iris



Fig 11

stroma in acute keratoconus with staphylococcus aureus ulcer (A) Extension of limbal vessels into the cornea with leakage at the apex of the vessels (B) Late phase with peripheral vessels as negative shadows in surrounding fluorescence and accumulation of fluorescein in margins of central oedematous cornea.



Fig 12

stroma in multiple pterygia showing (A) filling of straight arteries and branching vessels and (B) in venous phase extensive network of capillaries and draining veins with discrete leakage

### Multiple pterygia with keratoconjunctivitis

A 50 year-old woman with bilateral multiple pterygia and staphylococcal conjunctivitis five days after frequent instillation of antibiotics showed, in fluorescein angiograms straight arteries extending from the conjunctiva onto the cornea and a network of capillaries which was drained by thicker veins (Fig. 1). In the late phase vessel trunks appeared as negative shadows in diffuse fluorescence.

### Discussion

This is the first study to show the staining of the avascular corneal ulcer with fluorescein in the late phase of angiography. Simple marginal ulcers do not stain from the adjacent limbal vessels which leaked fluorescein. The ulcer might also stain from the limbal vessels in the late phase because even in a normal limbus (Mitsui et al. 1969) three min after the injection diffuse fluorescein was approaching the centre of the cornea. The possibility of fluorescein leakage from conjunctival vessels via the tear film to the ulcer could not be excluded in angiograms.

Any vessels in a previously normal cornea are due to neovascularization. In a study in acute complicated keratoconus and in Mooren's ulcer the corneal vessels decreased during vascularization and in corneal graft failures the vessels followed the immune response to the graft suggesting that the neovascularization was not the cause but the consequence of the corneal inflammation. The leakage of corneal vessels is a function of the degree of surrounding inflammation, compactness and may serve as an indicator of the amount of active disease underlying corneal disease (Kottow 1978). In this study in simple marginal corneal ulcers the adjacent limbus showed an early microscopic inflammation. In a chronic corneal ulcer, disciform keratitis, rosacea keratitis and Mooren's ulcer a progressive pannus with profuse leakage was seen. The tendency of fluorescein extravasation was much less in the pannus of sclerokeratitis and in gutter in rheumatoid arthritis as was seen by Kottow (1978) in sclerokeratitis in sclerouveitis.

Fluorescein angiograms showed fluorescein leakage of the iris vessels in iritis, irritative iritis during the active stage of simple and chronic corneal ulcers, disciform keratitis, Mooren's ulcer and in graft rejection. In the case of keratouveitis, iritis, vitritis and cystic macular oedema were seen as a general ocular inflammation associated with the corneal neovascularization.

### Acknowledgments

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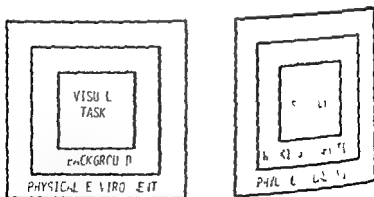
## Visual Requirements in Industry

During the last twenty years much effort has been concentrated on the determination of visual requirements for workers in various branches of industry. Wirt (1945) were the first to study visual performance in relation to job performance. In a large scale study they obtained statistical evidence to support the visual acuity for the successful performance of different industrial jobs. If visual and administrative work productivity fell when the corrected visual acuity was less than 20/40 when the corrected acuity at near was less than 70/30 and when muscle balance for near was abnormal. No influence could be detected for heterophoria at distance or of colour perception. Transport workers however were found to be more dependent on distant visual acuity on performance perception and on unimpaired binocular vision at distance. Unskilled workers were successful performers even when their corrected distant acuity was 20/40. Muscle balance and colour recognition were of no importance. In the future such standards are used as a guidance when pre-employment eye examinations are practised to place prospective workers in suitable jobs.

After all such standards for fitness in industry are difficult to establish and troublesome to collect data from a sufficient number of uniform workers and as a matter of fact rather few persons have substandard visual capacity.

Thus apparent correlation could be inconclusive because statistical observations are homogenous observations.

Execution of work is dependent on an interaction between the worker and the work (Fig. 1). The study of the job itself comprises a classification of the gross work taking in consideration the crucial details and the visual information available. Further qualities to be considered when the background of the work piece is to be described are the individual working place illumination with regard to both quality and quantity of light, glare, daylight factor and free view to



Furthermore the effect of noise heat and humidity must be taken into account. Of course the visual performance profile of the employee is mandatory to visual capacity but other important factors are the working posture and the psychological stimulation i.e. the relationship between skill and task. Maladjustment to work may cause stress and so may bad co-operation with fellow workers and workload.

Thus a job analysis is a complex matter and the influence of one sole factor e.g. visual capacity on productivity security or individual well being can only be evaluated if all other conditions mentioned are comparable and that is an unusual situation. Productivity studies are further jeopardized by the fact that modern production is often performed in groups with a shifting job routine. Thus an individual effort is difficult to evaluate.

### Present Study

This paper only summarizes some results from a more comprehensive report published elsewhere (Clausen et al. 1978). The electronic industry concerns soldering mounting print plates work repair and control and many of these processes present demanding visual tasks. This industry employs both younger and elderly workers and may be considered amenable for the study of visual problems. The field study was conducted by one academic engineer trained in ergonomics one occupational therapist, one academic lighting engineer one psychologist and one orthoptist. The visual test took place in a mobile specially equipped cabin placed near the workshop.

The study involved 162 employees and age varied between 18 and 60 years. The average was 39 years. Two-thirds were women. Several cross references were made in attempt to show relation between observations recorded by the various examiners. The total amount of persons and the heterogeneous data composition never invalidated many statistical evaluations. Thus the study must be considered a pilot study suggesting tendencies to be proved in studies on a larger scale. The tests for visual acuity and for near vision revealed few facts of importance. 93% of the subjects had a corrected visual acuity of 6/6 and 97% could read a point print at a distance of 40 cm. This leads to the conclusion that a screening of these basic visual functions would have been of limited value in this plant.

One could imagine that visual unfit workers had left the industry spontaneously after a short period. Such a natural selection could not be demonstrated. 38% had uncorrected visual acuity below 6/6 a figure that agrees with the results of the aforementioned screening tests. 29% of 162 had heterophorias exceeding +1 pd and -9 pd read on Maddox Wing test. Nor does this figure indicate any selection.

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## PRESENILE CATARACT

A follow up investigation with special reference  
to occupational aspects

BY

DANUTA MARUSZCZAK HANNE JENSEN  
and ERNST GOLDSCHMIDT

A retrospective investigation was carried out of 100 patients below the age of 60 years operated for presenile cataract

The material has been divided into three occupational groups depending on the visual requirements

The complaints prior to and after operation have been related to the patients' ability to retain their employment. Of 8 patients who were still employed, only 40 were able to return to their former occupation after the first cataract extraction

The final result was that 62 patients could continue their former work, 1 changed their occupation due to aphakia problems while four had to retire

The vision in the fellow eye, the type of work and the optical appliances have been found to be of primary importance

It is concluded that operation for unilateral cataract is inadvisable if the vision of the fellow eye is good and contact lenses cannot be used, that the interval between operations for bilateral cataract should be as short as possible and that the use of contact lenses is essential

**Key word:** presenile cataract - occupational aspect - correction of aphakia - simultaneous bilateral cataract extraction



g advice to patients suffering from presenile cataract in respect of the choice of time of operation is difficult. In cases of occupational problems the ophthalmologist will often be subject to a certain amount of pressure from the patient. It must therefore be of interest to determine whether early operation complies with the expectations of the patient, b) permits retention of the patient and c) whether the operative method and various forms of correction of astigmatism influence the results

## Material

During the period 1 4 1972 to 31 12 1977 143 patients below the age of 60 years were referred to the Ophthalmological Department of the Odense University Hospital for operation for presenile cataract. All those patients were recalled to the department in the latter part of 1978 for a follow up examination. Forty three did not attend of these 9 were dead, 13 had moved to other parts of the country, 10 in poor health and 11 did not react to repeated inquiries. The final material thus consists of 100 patients, 60 men and 30 women, the age and sex distribution illustrated in Table 1. The average age of the men was 54.5 years and of the women 53 years. Forty four men and 23 women were operated on both eyes, the remaining 93 patients on one eye only. Thus 167 operations are included in the material.

## Methods

Information was obtained from the case records regarding preoperative visual acuity, the operative method as well as complications during admission.

Table 1  
Age and sex distribution of 100 patients operated for presenile cataract

Age (years)	Male	Female
31-40	1	3
41-50	9	7
51-60	50	20
Total	60	30

Table II  
Duration of interval between operations in 6  
cases of binocular cataract extraction.

Duration (months)	Male	Female
1	2	1
2-6	9	3
7-12	10	9
13-24	10	5
25-36	7	3
37-48	2	1
49-60	2	1
Total	44	23

The follow up examination included the following

1. An interview with the patient regarding the occupational activities and length of sick leave
2. Information regarding the correction of the aphakia (this was part of the patient's optician)
3. A physical examination including visual acuity, biomicroscopy or ophthalmoscopy, evaluation of binocular function by means of Worth (15) and titmus stereo test

The follow up examination was carried out 6-72 months after the operation on eye one with an average observation time of approximately 3 years.

Table II shows the number of months between the operations on the binocular operation. Only one patient was subjected to operation on both during the same admission.

The preoperative visual acuity in the better eye is shown in Table III. It appears that 50% had a visual acuity of 6/12 or more at the time of operation.

Table III  
Preoperative visual acuity in fellow eye in 100 patients

Visual acuity	Male	Female	Total
6/6	14	4	18
6/9-6/12	23	9	32
6/18-6/24	19	13	32
6/36	9	3	12

ative method

eyes were subjected to intracapsular operation with corneo-scleral incision and peripheral iridectomies the lens was extracted employing the cryotechnique. In 3 cases enzymatic zonulolysis was used. The extracapsular operative technique was chosen in 29 cases. The average period of hospitalization was 10 days for operation on eye one and 8 days for operation on eye two.

## Results

Visual improvement is shown in Table IV. The preoperative visual acuity of the 167 operated eyes is related to that immediately postoperatively (approximately 8 days) and the vision at the time of follow up. As expected, a pronounced improvement in visual acuity is seen, and 90% had  $\geq 6/12$ , without any significant difference between intra- and extracapsular operation. However, a vision of 6/6 is seen significantly more frequently in those subjected to intracapsular operation.

The operative and postoperative complications are shown in Table V. The most frequent complication was transient ocular hypertension from day 2 to 3 postoperatively. It was necessary to re-admit 8 of 29 patients subjected to extracapsular operation for discussion. However, this was not required in any of the seven cases of intracapsular rupture.

At the time of the follow up examination, the vision was  $< 6/18$  in 12 (8.7%) of the eyes operated intracapsularly and in four (13.8%) of the extracapsularly operated eyes. The causes of the reduction in vision are shown in Table VI.

Table IV  
Visual results in 167 eyes

Visual acuity	Preoperative	Postoperative	
		8th day	Follow up
<i>Intracapsular extraction</i>			
6/6		41	102
6/9 -6/12	1	58	24
6/18-6/24	15	31	4
≥ 36	122	8	8
<i>Extracapsular extraction</i>			
6/6		8	15
6/9 -6/12		6	10
6/18-6/24	4	8	1
≥ 36	95		3

Table 1  
Operative and postoperative complications in 107 eyes

Complication	Intracapsular extraction		Extracapsular extraction	
	Male (92 eyes)	Female (15 eyes)	Male (11 eyes)	Female (1 eye)
Broken vitreous	1	1		
Ruptured capsule	5	2		
Ocular hypertension ( $>25$ mmHg in 2-6 days)	31	9	5	1
Hypaemia	2	1		
Vitreous haemorrhage	1	1		
Cystoid oedema	2			1
Retinal detachment	5			1

#### Occupational aspects

An attempt has been made to evaluate the occupational aspects, both pre- and postoperatively.

The material has been divided into three occupational groups, depending on visual requirements. The distribution is shown in Table VII.

The results of the investigation are shown in Tables VIII and IX, a comparison being made between those operated on one eye only and those subjected to bilateral operation.

Table VI  
Ocular pathology in 19 intra- and 4 extracapsular  
operated eyes with  $\leq 6/18$  visual acuity at the time  
of follow up examination

Finding	Intracapsular extraction $n=19$	Extracapsular extraction $n=4$
Vitreous haemorrhage	2	-
Secondary cataract	1	2
Retinal detachment	3	-
Cystoid oedema	1	1
Corneal opacity	2	1
Diabetic retinopathy	2	-
Amblyopia	1	-
Total	19	4

*Presenile Cataract*

**Table VII**  
Occupational activities in 100 patients

	Occupational category	Male		Female	
		Unilateral aphakia	Bilateral aphakia	Unilateral aphakia	Bilateral aphakia
1	High visual* requirements	6	19	5	3
2	Moderate visual** requirements	4	9	11	8
3	Close work***	9	12		1
4	Fine work			4	10
5	Heavy work	2	4	1	1

Skilled workers with fine work: drivers, ships officer etc.  
 Skilled and unskilled workers with heavier work: farm labourers etc.  
 Executive teaching or office work.

**Table VIII**  
Occupational and sick leave in 26 patients operated for unilateral cataract

	Occupational category						Total n = 26
	1		2		3		
	Male n = 5	Female n = 6	Male n = 4	Female n = 2	Male n = 9	Female	
<i>Operative</i>							
Sick leave	—	3	—	1	1	—	5
Change of occupation	1	—	—	—	—	—	1
Severe difficulties	5	2	4	1	8	—	20
No difficulties	—	—	—	—	—	—	—
<i>Non-operative</i>							
Sick leave	—	1	—	—	—	—	1
Change of occupation	—	1	1	1	—	—	3
Severe difficulties	—	—	—	—	—	—	—
No difficulties	11	3	3	1	9	—	27

Table IX  
Occupational and sick leave in 52 patients operated for bilateral cataracts

	Occupational category					
	1		2		3	
	Male n=19	Female n=3	Male n=9	Female n=3	Male n=19	Female n=1
<i>Preoperative</i>						
Sick leave	8	1	1	3	1	
Change of occupation	3	-	2	1	-	
Severe difficulties	8	2	6	4	11	1
No difficulties	-	-	-	-	-	
<i>After 1st operation</i>						
Sick leave	3	-	-	-	1	1
Change of occupation	3	1	3	1	1	
Severe difficulties	7	1	1	-		
No difficulties	2	1		7	8	
<i>After 2nd operation</i>						
Sick leave	1	-	-	-	1	1
Change of occupation	3	-	2	1	1	
Severe difficulties	-	-	-	-	-	
No difficulties	13	3	7	7	10	-

Preoperatively all 78 had difficulty in carrying out their work and was a contributory factor in the desire for operation. Seven of the 78 had wholly changed occupation and 19 had been on sick leave for up to 12 months prior to admission.

Postoperatively, i.e. after operation on eye one, there were no difficulties depending on the vision in the fellow eye, the type of work and the type of appliances. Only 45 of the 78 patients were able to return to their work without special problems after the usual period of convalescence.

A more thorough analysis showed that there is a relationship between the vision in the unoperated eye and the working ability postoperatively.

Of 26 patients (Table VIII) operated for unilateral cataract, 23 were able to return to their work after operation. The majority had good vision in the fellow eye and as it is shown later, accepted contact lenses. Among the 52 patients operated for bilateral cataract (Table IX) only 23 could after operation on the second eye continue in their former work without difficulties.

number of patients despite a successful operation on the first eye required a period of sick leave or were forced to change their occupation. This applies in particular to patients in occupation. This applies in particular to patients in occupational group 1.

10 men in occupational group 1 (Table IX) who required postoperatively sick leave (3 to 6 months) had poor vision in the fellow eye ( $\leq 6/24$ ). The period of time from operation of the fellow eye was from 2 to 9 months.

10 men and one woman in the same group 1 changed their occupations after a successful operation on eye one as they were unable to carry on work without ocular function. In these cases the vision in the fellow eye was also relatively poor ( $\leq 6/18$ ). Operation on the second eye was not carried out until after 11 to 36 months.

In group 2 where the requirements to vision were less exacting no patient needed sick leave between the operations but 3 men and 1 woman changed their occupation due to the aphakia problems; the vision in the fellow eye was also  $\leq 6/18$ . These patients waited between 10 and 24 months for operation on the second eye.

In group 3 3 patients who had to have sick leave or change their work also had poor vision in the fellow eye. The situation changed considerably after the operation on eye two (cf. Table IX bottom).

The final result was that 62 patients from the total group of 78 still employed could continue in their former occupation. 12 changed their work due to aphakia problems while 4 had to retire.

The housewives with bilateral cataract had severe problems after operation of eye one and needed assistance in housekeeping. After the second operation no assistance was necessary.

Table X  
Glasses or contact lens wear related to visual acuity of phakic eye in 100 patients

Visual acuity in fellow eye	Male			Female		
	No of patients	Glasses	Contact lens	No of patients	Glasses	Contact lens
6/6	14	2	19	4	1	3
6/9 - 6/12	23	5	17	9	6	3
6/18 - 6/24	19	13	4	13	8	4
$\leq 6/36$	9	8	1	9	8	1
Total	65	28	34	35	23	11

Free men and one woman had no correction for the aphakia

Table VI  
Contact lens wear related to age in 100 patients

Age (years)	Male		Female	
	No of patients	Contact lens	No of patients	Contact lens
31-40	1	1	3	1
41-50	9	5	1	3
51-60	52	28	23	5
Total	62	34	27	11

A number of patients were only able to retain their occupation if they were permitted to drive a vehicle. Of 61 patients with driving licence who had previously driven a car 31 stopped driving prior to the operation. 14 have resumed driving after operation.

#### Selection of correction for aphakia

Table V shows the correction for the aphakia as related to the visual acuity of the eye. Four patients had no correction for the aphakia and many were fitted for correction 3-6 months after operation. It can be seen from the table that 34 (32.3%) and 11 women (31.5%) had contact lenses but of these 3 women had discontinued the use mainly due to irritation and difficulty in the insertion and removal of the lenses.

There is a relation between visual acuity of fellow eye and choice of correction for aphakia. Of the 50 patients operated for unilateral cataract with 66-61 in the fellow eye 70% employ contact lenses.

In the other group of 50 patients with visual acuity worse than 1/4 in the unoperated eye only 20% wear contact lenses.

The contact lens frequency is somewhat lower in women but among those in an occupation the frequency is almost as high as in men.

Table VII  
Contact lens wear related to occupational category

Occupational category	No of patients	Contact lens	%
1	33	21	61
2	23	5	22
3	22	14	64



fourteen patients with glasses and visual acuity 6/6-6/12 in the fellow eye had not benefited from the operation and the majority used the phakic eye operatively.

The age distribution of the patients who primarily employed contact lenses is shown in Table XI. The motivation for employing contact lenses appears to be somewhat stronger in men than in women and in younger patients. Table XII shows the use of contact lenses in the various occupational groups and it may be seen that patients with high visual requirement or involving much close work use contact lenses far more frequently than patients with moderate visual requirements.

#### Binocular function

Binocular function was studied by means of the Worth 4 DOT test and the Titmus stereo test. The results are shown in Table XIII from which it can be seen that the binocular function was considerably better in those employing contact lenses than in those using spectacles.

Table VIII

Stereo-acuity in 19 unilateral aphakes wearing contacts and 66 bilateral aphakes wearing contacts or glasses

	Sex	No of patients	Full stereopsis No of patients	%
<i>Unilateral aphakes n = 19</i>				
Contacts	Male	15	7	
	Female	4	3	
Total		19	10	52.6
<i>Bilateral aphakes n = 66</i>				
Glasses	Male	27	8	
	Female	19	7	
Total		46	15	32.6
Contacts	Male	16	12	
	Female	4	2	
Total		20	14	70

## Discussion and Conclusion

The present investigation which includes 63 men and 33 women between 40 and 60 years of age has been carried out retrospectively. During the period under study clear lines of direction have been laid down regarding the selection of the operation and the optimal period between the operations on the two eyes.

The investigation shows that the final vision is comparable regardless of whether intra- or extracapsular technique is employed and that there are no postoperative complications. Re-admission for discussion of the problem is considered an extra strain by the patients even though the period of hospitalization is short.

Occupational problems have been a major factor in the desire for operation. A number of patients have changed their occupation prior to the operation. Some have been granted sick leave while some others who have worked until the day of the operation found that this was only possible due to the fact that they were able to reduce the speed at which they worked or who had sympathetic employers or helpful colleagues.

A contributory cause to the postoperative problems is undoubtedly the fact that the patients expect cataract extraction to solve all their visual problems. The same opinion is undoubtedly held by many colleagues at work. Employers and co-workers. The long period of waiting between operations had been a further problem. A waiting time should be avoided. In this connection it should be emphasized that the patients comprising the present material have been referred to the department by 10 different practicing ophthalmologists. After discharge the patients are asked to consult them; these ophthalmologists also determined the type of correction required for the aphakia.

Unilateral cataract extraction with a well-preserved vision in the fellow eye does not appear to give any greater problems neither pre- nor post-operatively. The patients adapt themselves to the situation also in respect of their employment. The majority are able to return to their previous trade or profession after the usual period of postoperative sick leave. This group of patients seems to be particularly inclined to use contact lenses and do not have any greater problems with them including insertion and removal. The patients who cannot or will not use contact lenses have little or no benefit from the operation and therefore the operation is deferred as long as possible. The group of patients who present with bilateral cataract development in both eyes are those with the greatest problems. Unilateral cataract extraction gives an improvement in vision from which the patients derive limited beneficial effects. Only a few are able to employ contact lenses and these have serious problems in respect of their occupation particularly those who are requiring especially good eye sight. The situation does not improve until after the second operation.

then performed on the second eye and the investigation clearly demonstrates the interval between operations has been too long. The previous assumption operation of the fellow eye should be deferred until the patient has been corrected for the aphakia and has become accustomed to the use of this does not appear to be reasonable especially for those patients who are still employed. In recent years there has also been an increasing number of ophthalmologists who have advocated the use of simultaneous binocular cataract extraction (Jardine 1971, Nissim & David 1977, Suchting 1973).

The risk of postoperative bilateral intraocular infection seems to be overestimated. Jardine performed 580 simultaneous cataract extractions and there were no cases of intraocular infection but one eye only in each case. Nissim reported no cases of monolateral endophthalmitis in 676 bilateral cataract extractions in one session.

Another possibility is to carry out extraction with an interval of a few days during the same hospital admission. This is advantageous for the patient, as it requires a considerably shorter total period of sick leave and is less expensive for the community.

The patient should be given preliminary correction for the aphakia a few days before the operation in cases of bilateral cataract extraction carried out during the same hospital admission.

As many patients as possible should employ contact lenses and it is also important that the patients are given correction suitable for their particular work.

None of the patients examined had intraocular lens implants but from the experience gained from elderly patients with clip lenses it is obvious that this form of correction of the aphakia is superior to any other.

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## Results

## Industrial measurements

The flux densities and the intervals within which they normally vary at the sites ordinarily occupied by the operator are shown in Figs 1 and 2.

The welding processes generated magnetic fields with frequencies of 50 Hz and with flux densities within the range  $10^{-4}$ – $10^{-3}$  T which can be compared with the earth magnetic field which varies from about 0.03 to about 0.05 mT (Fig. 1).

Considerable variations may occur due to the current strength and the proximity to metal bodies etc. Nevertheless the values given may be regarded as characteristic of the different methods respectively.

Among the electric steel processes the ladle furnace gave a rather high flux density in the proximal zone. Particularly high values were recorded in induction heaters. For induction heaters both magnetic flux density and frequency may be high (up to 10 kHz) implying a high energy density of the electric arc compared to other electrical processes in the frequency range dealt with in this work (Fig. 2).

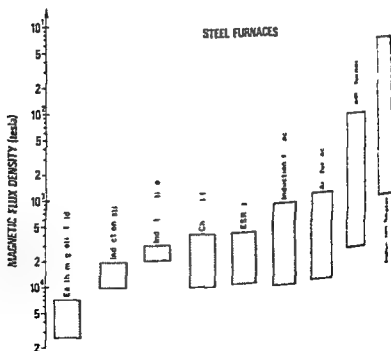


Fig. 2. Magnetic flux densities near (at the site of the operator) different steel-making processes (induction refining).

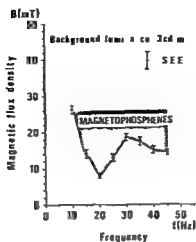


Fig 3

Magnetophosphene threshold curve at a background luminance of  $3 \text{ cd/m}^2$ . Phosphenes are seen at magnetic flux densities above the threshold curve. 10 volunteers.

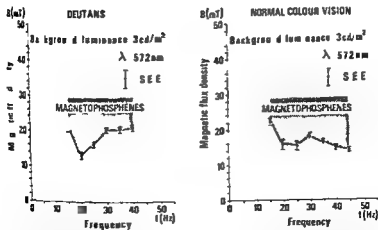


Fig 4

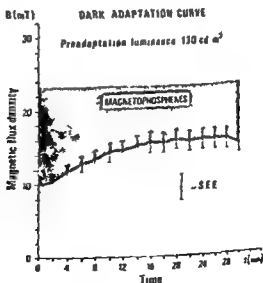
Magnetophosphene threshold curve for deuterans and volunteers with normal colour vision at  $572 \text{ nm}$  and  $3 \text{ cd/m}^2$  background light, 9 and 6 volunteers respectively.

# Magnetophosphenes

The threshold values for magnetophosphenes at the different background luminance levels were determined as in Fig. 3 in which the magnetic threshold values are plotted as a function of the frequency of the magnetic field. A threshold value is defined as the magnetic flux density at which the phosphene sensation disappears after having been seen at higher flux densities. Every threshold value is the mean of two determinations.

The threshold values varied with the frequency of the magnetic field and the luminance level of the background light. In virtual darkness there was a sensitivity maximum at 30 Hz. At higher luminances this sensitivity maximum shifted towards lower frequencies (20–25 Hz) and a second maximum was observed at about 40–45 Hz. The maxima were separated by a local sensitivity minimum at 30–35 Hz. The local minimum at 30 Hz was clearly seen at 3 cd/m<sup>2</sup>. At the highest luminance (130 cd/m<sup>2</sup>) the curve was rather flat above 20 Hz.

The thresholds (at 3 cd/m<sup>2</sup>) for the colour defectives differed from those for the volunteers with normal colour vision. The threshold curve for the normals showed a sensitivity maximum at 20–25 Hz and a local sensitivity minimum at 30–35 Hz. Thereafter the sensitivity increased again (Fig. 4). The maximum and the local minimum were stationary at the same frequency also when the wavelength of the background light was changed. The threshold curves for the deuterans did not differ from those of the normals.



F. 5

Threshold values for magnetophosphenes determined during dark adaptation at the frequencies 20, 30 and 45 Hz. 8 volunteers.

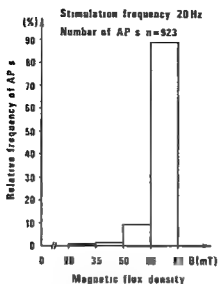


Fig 6

responses in the ganglion cell layer as function of the magnetic flux density at 20 Hz the first second after switching off the field (AP's action potentials)

sensitivity minimum and the following sensitivity increase for the wavelengths (Fig 4) and 531 nm while they were present at 443 nm

The results of the dark adaptation studies is shown in Fig 5 With increasing time in the dark the threshold values also increased

ganglion cell activity induced by ELF magnetic fields

The responses from the ganglion cell layer varied to a great extent with the magnetic flux density and the field frequency In accordance with the responses to 50 Hz, the responses to magnetic field stimulation occurred only at ON and/or OFF

The variation of the responses from OFF-cells with the magnetic flux density the first second after switching off the field is exemplified in Fig 6

## DISCUSSION

The purpose of the industrial measurements was to establish the range within which the magnetic flux density could conceivably vary in different energy consuming electrical processes in the welding and steel industries With this knowledge it should be possible to simulate corresponding fields under laboratory conditions and also to describe and predict possible physiological effects

The aim of the magnetophosphene studies was to determine threshold values of phosphenes and to shed some light onto the problem of phosphene generation. It was discovered that the electromagnetic fields interact with the visual system in a reproducible way. ELF fields of moderate flux densities found in industry can induce magnetophosphenes. The relations between the threshold values of magnetophosphenes on one hand and magnetic field frequency, wavelength, wavelength of the background light, colour vision properties and retinal adaptation level on the other hand seem to reflect that the phosphenes are generated in the same retinal channels that are normally propagating signals induced by light of different qualities and that the sensitivity of different channels is related to the frequency of the stimulus. We have not found evidence of any damage to the visual system of short term exposures to these types of magnetic fields. However, the results do not permit any definite conclusions as to possible effects of long term exposures in welding and electrosteel industries.

As an effort to further explain the origin of the magnetophosphenes, we made intraretinal measurements of the electrical activity of the ganglion cells. The results from this part of the study clearly prove that the retina is excited by the magnetic field stimulation. They also indicate that the phosphenes are generated in the inner retina and that there are several similarities between the responses in the ganglion cells to light and to ELF magnetic fields. Possible mechanisms behind the magnetophosphenes will be further discussed in a following paper (Löwsund *et al.* 1979).

Our results may be of guidance when formulating threshold limit values for flux densities for ELF electromagnetic fields in the industry.

### Acknowledgments

Miss Birgitta Svensson at the Department of Biomedical Engineering, Linköping University is acknowledged for skilful assistance.

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*Short Communication*

PERFORATING EYE INJURIES CAUSED BY OCCUPATIONAL  
ACCIDENTS TREATED AT HELSINKI UNIVERSITY  
EYE HOSPITAL IN 1970 TO 1977

BY

MATTI NIIRANEN

192 consecutive perforating eye injuries caused by occupational accidents in 1970-77 were studied. They represented 40% of all perforations treated during that period at the Helsinki University Eye Hospital. The results were compared with the previous series from this hospital from 1920-29 and 1930-39.

92% of the patients were males, a slight decrease from the previous series. The age group 16-25 years was largest (30%). Previously the next group 26-35 years had been the largest.

The proportion of workers in building construction had increased remarkably and comprised 26.6%. Another large group were metal workers. Agricultural work had lost its importance as the cause of perforations compared with the previous decades.

The commonest cause of accident was still hammering. Especially metal splinters were important causes in both the present and in the previous series.

Prognosis was much better in the present series than earlier. 81% of patients achieved vision of 0.5 or better, as compared with 40% in the twenties and 20% in the thirties.

*Keywords:* perforating eye injuries - occupational perforations - level of vision - perforations - prognosis of perforations

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## CHLOROQUINE KERATOPATHY IN CHLOROQUINE WORKERS AFTER TOPICAL DUST EXPOSITION

### A Case Report

BY

LEIF STEENE ERIKSEN

A report of acute corneal changes in workers employed in the chloroquine industry is presented. The workers were exposed to chloroquine phosphate dust in the manufacture of chloroquine tablets and the corneal alterations were similar to those described after long continued systemic chloroquine therapy.

*Key words:* cornea - chloroquine - keratopathy - dust exposition - man.

Chloroquine is a quinoline derivative with a coplanar fused ring system. It was introduced as an antimalarial drug in 1945 and is today the drug of choice for the treatment of malaria. Since 1950 it has assumed increasing prominence in the treatment of chronic rheumatoid arthritis and lupus erythematosus. Long continued systemic use of this substance is known to produce side effects, the most important of which are ophthalmological. Two distinct types of ocular side effects are known: one affecting the cornea and the other the retina.

Chronic systemic treatment with chloroquine induces morphological alterations of the cornea in a proportion of cases. Chloroquine is transported by the blood stream to the corneal epithelium via the perilimbal vascular plexus or via the tear film and tear fluid. The epithelial alterations are very characteristic in appearance and fairly constant in localisation. The most characteristic features are slightly wavy undulated lines running horizontally at the junction of the lower and middle thirds of the cornea. This fairly constant localisation and peculiar shape is thought

to be due to a form of massaging effect exerted on the eyelids by the eyelids.

Chloroquine disturbs the normal phospholipid metabolism of the corneal epithelium and the alterations in the cornea seem to be part of a generalized phospholipidosis (Seiler et al 1977). Typical intracellular deposits are found in the corneal epithelium cells. Similar deposits occur in all tissues with high phospholipid turnover. It has been reported that about 30-40% of the patients on long-term medication with chloroquine develop these corneal changes (Saxena et al 1977). There is no direct relationship between the total dose used and the extent of the corneal changes; differences are presumably due to an individual variation in drug response. The corneal changes do not affect the visual acuity; they are reversible and disappear after the treatment is discontinued.

### Case Report

A 45-year-old man previously employed in a furniture shop damaged in a fire in a producing factory. The first day in his new job he began to manufacture these tablets. This type of tablets had not been manufactured in the factory for the years before. The workers had complained of discomfort in the eyes. During the first day of the tablets the man was continuously exposed to chloroquine phosphate dust. He wore a mask to cover his mouth and nose but had no eye protection. Irritation of the eyes was present from the first day. During the next few days his eyes became red and his vision became blurred. After four days he was referred to the eye department. At punctate erosions of the corneal epithelium were found. After three days of local therapy a control examination was performed. The epithelium had healed but the changes in the cornea similar to those described after long-term treatment were still present. Except for the corneal alterations the entire anatomical and functional examination of the eyes were normal. Six weeks later the corneal changes had disappeared. The employee was in the same production and also complained of eye irritation. He was exposed to the dust to a lesser extent and was not examined in the eye department.

Five months later there was a new production of chloroquine tablets. The eye irritation was reduced and the workers used spectacles to protect their eyes. The spectacles were not used continuously throughout the day. Our patient had no red and irritated eyes. Also on this occasion he had typical chloroquine keratopathy. Examination after five days in the work. Two other workers were examined in the production. They had the same symptoms. On examination one was found to have chloroquine keratopathy.

### Discussion

A report has been given of acute corneal changes in workers employed in the chloroquine industry; the changes consisting of deposits in the corneal epithelium. The deposits developed after topical dust exposition and the changes were reversible.

as to those described after long-continued systemic chloroquine therapy. This is of interest from an ergo-ophthalmological point of view. Mann (1947) described similar corneal changes in quinacrine (Atabrine) workers after local dust exposition. Under controlled circumstances essentially the same changes were produced in animal eyes by insufflation. Side effects are reported during clinical treatment with almost all drugs. In the production of potential toxic drugs considerable side effects can be due to direct contact with the eyes. It is therefore important to protect the eyes and reduce dust exposition.

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## NEW ASPECTS ON THE HEREDITY OF OPEN ANGLE GLAUCOMA

By

TORD JERNDAL and ANDERS LIÖD

Open angle glaucoma is known to be inherited but detailed knowledge of the primary genetic factor and its mode of inheritance are lacking. Because both the glaucomatous and non glaucomatous members in afflicted families it has been established that premorbid goniodysgenesis inherited in an autosomal dominant mode plays a decisive role for the eventual development of glaucoma. Goniodysgenesis has been documented not only in late congenital glaucoma but also in late congenital glaucoma (senile cataract and glaucoma simplex) and in families with pigmentary glaucoma and exfoliation glaucoma.

In addition there is evidence that goniodysgenesis is the morphologic prerequisite of the high elevation of the IOP on provocation with pupal water. The article contains a short survey of the literature and presents three families with intrafamilial variations of open angle glaucoma based on a defined genetic trait in goniodysgenesis.

**Key words:** genetics - glaucoma - goniodysgenesis - heredity - open angle glaucoma - pedigree

It is well known that open angle glaucoma may run in families as an inherited disorder. In textbooks on heredity François (1961) and Waardenburg et al. (1964) is stated that infantile congenital glaucoma (buphthalmus) is recessively inherited but juvenile glaucoma and glaucoma simplex are dominant.

In contradistinction Becker (1963) and Arnall (1964) in a number of papers designed a genetic model which according to their conclusions speaks in favour of a recessive mode of inheritance of simple glaucoma.

Unfortunately the entire subject is most unclear due to the lack of uniform criteria and unequivocal terminology.

to be able to draw valid conclusions from studies of glaucoma pedigrees the following demands must be met with

The primary genetic trait should be searched and if possible defined

All members glaucomatous as well as non glaucomatous should be properly examined with regard to the genetic trait

Criteria for the diagnosis of glaucoma must be defined

The type of glaucoma should be classified with a careful gonioscopy

If these four demands were to be fulfilled approximately 90 per cent of the material on hereditary glaucoma would be disqualified. The remaining 10 per cent, however, lend themselves to an analysis from which the following conclusions can be drawn

#### Infantile congenital glaucoma

The important primary point of view is to recognize infantile congenital glaucoma as the consequence of goniodysgenesis. Andersson (1939) Hlavskens (1950) Worst (1966). From this starting point it is no neckbreaking step to assume that infantile congenital glaucoma is a severe form of dysgenesis of the drainage pathways, a milder form of which may result in juvenile or adult glaucoma.

The first pedigree (Fig. 1) although small demonstrates this in a clear manner (Andal 1968). The dominant trait - goniodysgenesis - is demonstrated in six members. Glaucoma has so far developed in three of them at the age of three months, 10 years and 46 years respectively. This pedigree has a strong resemblance to that published by Gillespie (1963) with the title "Congenital glaucoma, juvenile glaucoma, chronic simple glaucoma, all in one family."

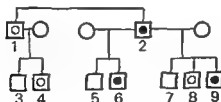
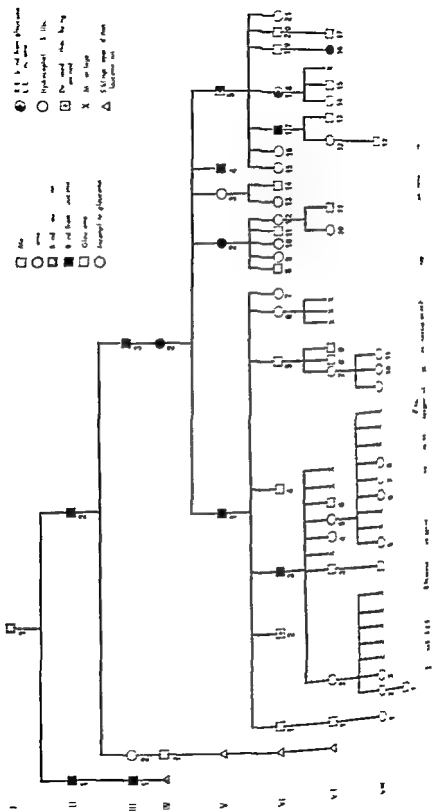


Fig. 1

Andal (1968). Member No. 9 developed infantile congenital glaucoma at 3 months of age. Member No. 6 has half-brother developed late congenital glaucoma at 10 years and their father No. 1 developed glaucoma at 46 years. They all had distinct goniodysgenesis. Moderate goniodysgenesis also found in members 1, 4 and 8.





# juvenile glaucoma

A poorly defined class of glaucoma is characterized by autosomal dominant goniodysgenesis of the anterior ocular segment (Berg 1932 Weatherill & Hart 1953) and a poor prognosis on medical therapy.

The pedigree reported by Berg was re-examined in 1970 by Jerndal (Fig 2) who in addition to the previously reported kerato- and irido-dysgenetic signs also observed goniodysgenesis in the glaucomatous members. There are at least three members who developed a verified infantile congenital glaucoma before one year of age, the rest of the glaucomas appeared between 1 and 45 years of age.

# simple glaucoma

As seen above the same dominant dysgenesis may result in a glaucoma from early infancy to the forties. A pedigree from the monograph of Jerndal et al (1978) gives more information regarding the role of dominant goniodysgenesis (Fig 3).

Three generations are afflicted and the glaucomas were discovered between 45 and 55 years of age except for case III 1 - a myopic male whose glaucoma was diagnosed at 32 years. All three glaucomatous members examined by us had typical goniodysgenesis.

III 4 in addition had exfoliation phenomena and III 1 the classic clinical picture of pigmentary glaucoma.

Thus this family at first sight appeared to have a dominant simple glaucoma but closer examination demonstrated dominant goniodysgenesis combined with exfoliation and pigmentary syndromes.

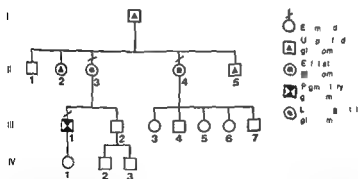


Fig 3

Jerndal et al (1978) Pedigree with dominant glaucoma presenting late congenital glaucoma, exfoliation glaucoma and pigmentary glaucoma all with typical goniodysgenesis.

## Discussion

A summary of pertinent literature and the presented pedigrees make it clear that open angle glaucoma may exist as a dominant disorder and that it may occur in early infancy and in fact lacks an upper limit. The primary process is evidently a maldevelopment of the indo-corneal angle - goniodysgenesis - as discussed and highlighted with scanning electron microscopy by Jerndal (1978). If the dysgenetic changes are pronounced infantile or juvenile glaucoma may ensue. With less advanced goniodysgenesis additional factors may cause a gradually impaired outflow and eventually precipitate glaucoma because of several such factors i.e. the pigmentary liberation syndrome, the exudative syndrome, steroid provocation and hyphaema.

Many ophthalmologists have doubted that congenital glaucoma is a disease with a delayed onset of elevated IOP and glaucoma late in life. It is, however, a clinical experience that congenital anomalies can cause late functional disease e.g. stenosis of the renal artery and arterial hypertension, stenosis of the aorta of Sclera and hydrocephalus, congenital cystic kidneys and renal failure.

Another interesting finding by Jerndal & Munkbil (1978) is that the exudative high tensional response on provocation with topical steroids is not congenitally inherited and appears correlated to the presence of goniodysgenesis.

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## CONTRAST SENSITIVITY IN MACULAR DISEASE USING A SMALL-FIELD AND A LARGE FIELD TV SYSTEM

BY

JOHAN SJÖSTRAND

The spatial contrast threshold for a sinusoidal grating of various spatial frequencies generated with a small field or a large-field TV display was tested in normal subjects and in patients with macular disorders.

Impairment of contrast sensitivity (reciprocal of contrast) was observed in different maculopathies investigated. Attenuation of the high and middle frequency ranges was an early finding in macular disease whereas attenuation including the low frequency range was observed in more advanced maculopathies. Comparison of results obtained using the small (14°) or largest (6-24°) TV system demonstrated a field-dependence of the contrast sensitivity attenuation in localized macular disorders. In more widespread lesions of the posterior pole a contrast attenuation over the whole frequency range was found also with the largest (24°) stimulation field used.

The study of the contrast sensitivity function supplements the standard acuity measurements in quantifying the visual loss for objects larger than the resolution limit. I conclude that contrast threshold measurements are not only useful for describing visual loss but also for tracking progression of the disease.

The findings provide some additional insight into the visual disability and quality of life of patients with a macular disorder. However the definition of contrast sensitivity measurements for diagnosing different visual disorders is still lacking.

**Key words:** contrast sensitivity - maculopathy - spatial frequency - acuity

The commonly used acuity tests give information about the visual resolution for line targets at high contrast. The ability to see larger targets at lower contrast is of equal importance and is usually not tested in clinical practice.

In clinical practice we know however that patients sometimes complain of deterioration of low-contrast vision even though their visual acuity is within normal limits. A more general description of the visual system is obtained by studying the contrast sensitivity function, i.e. the reciprocal of threshold contrast for a range of spatial gratings that vary in spatial frequency (for review see Campbell 1974 or 1974). By the contrast sensitivity it is possible to study visual functions not included in the acuity test.

The aim of the study has been to investigate the contrast sensitivity function in maculopathies and the interest has been focused on the following questions: Contrast sensitivity measurement a useful additional clinical test in the diagnosis and follow up of macular disorders?

## Material and Methods

Subjects

10 normal controls (age 19–63 years) and 22 patients (age 19–78 years) with macular disease of different types took part in this study.

### Small field TV system

Sinusoidal gratings were generated on a television display as previously described by Frisén & Frisén (1977). The television monitor was masked to subtend  $1.4 \times 1.4$  degrees of visual angle at the eye when viewed at a distance of 0.5 m. A white illuminated mask which subtended approximately  $2 \times 2$  degrees surrounded the TV screen. Spatial frequency (number of cycles per degree of visual angle) was varied between 1.5 and 38 cycles/degree. Contrast (defined as  $(L_{\max} - L_{\min}) / (L_{\max} + L_{\min})$  where  $L_{\max}$  and  $L_{\min}$  are the maximum and minimum luminances of the sinusoidal pattern) was varied in 2 dB steps in the 0.63–0.0001 range. The space average luminance of the gratings was 135 cd/m<sup>2</sup>.

Contrast sensitivity (reciprocal of contrast threshold) was determined monocularly with correction and natural pupil. Seventeen frequencies were explored twice in a random order and the contrast threshold determined by raising the contrast from a sub-threshold level at a selected spatial frequency until a pattern was just visible by the patient.

### Large field TV system

The TV monitor used in the large field system was masked to subtend  $6 \times 6$  degrees of visual angle at the eye when viewed at a distance of 2.5 m. The TV monitor was surrounded by a white screen and by illumination of two daylight tubes a uniform background luminance of 100 cd/m<sup>2</sup> was obtained. The space average luminance of the grating was 83 cd/m<sup>2</sup>. The spatial frequency was varied between 0.5 and 30 cycles/degree. The pattern contrast of the sinusoidal grating was varied in 2 dB steps in the 0.6–0.001 range.

By changing the distance to the monitor the size of the TV screen was increased to  $9.4 \times 9.4$  degrees of angle at a distance of 0.6 m. The size of the retinal images (using the field size of 6 and  $9.4$  degrees respectively) is illustrated in Fig. 1. The test distance was controlled by using a support and refraction was optically corrected for the distances used. The test procedure was as described above for the small field system.

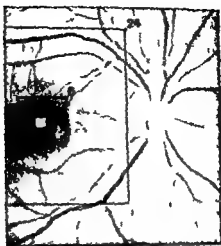


Fig 1

A fundus picture of a normal control with a schematic drawing of the retinal image on a TV screen which subtended 1.4 (□) 6 and 24 degrees of arc.

## Results

### Normal subjects

**Small field TV system** The contrast sensitivity curves (CSC) i.e. the contrast sensitivity plotted as a function of the spatial frequency of the sinusoidal gratings had a bell shaped form when tested for subjects with normal vision no previous eye disease and ophthalmoscopically healthy eyes (Sjostrand & Frisén 1977) in agreement with previous studies (Schade 1956 Campbell & Robinson 1968). The curves showed a high and low frequency attenuation with a peak sensitivity between 3 and 15 cycles/degree (Figs 2-4). Our CSC curves in normal controls using the small field TV system (1.4°) differ from those obtained using the large field TV system (6-24°) and from those previously reported in that our subjects' peak sensitivity occurred at a higher spatial frequency.

**Large field TV system** The contrast sensitivity curves of 10 normal controls obtained using the large field TV system had a much broader peak with a peak response varying from 1 to 8, 1-10 and 2-12 cycles/degree for a field size of 6, 19 and 24 degrees respectively (Figs 7 and 8). A comparison of the peak frequency obtained for normal controls measured on both the small field (1.4°) and the large field TV system masked down to 1.4° (see legend Fig 7) showed a mean difference of 0.5 cycles/degree for the two systems used (cf Figs 2 and 7). The difference in peak frequency therefore presumably reflects the mask and the surroundings and the luminances (cf Estevez & Cavonius 1976).

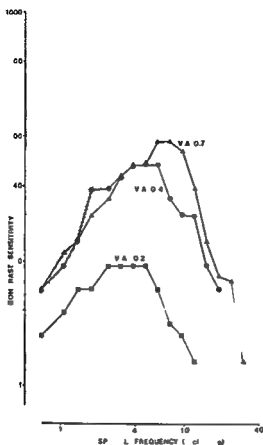


Fig 2

SC (1.4) in normal controls (dotted area indicates range for ten normal subjects aged 9 and 61 years) and three representative cases of different stages of *senile macular degeneration* (solid lines). Case 1 A ( $\Delta$  female 65 years-old) had moderate macular changes and VA 0.7 in case 1 L ( $\bullet$  male 46-years-old VA 0.4) changes were more advanced and in case 1 L L ( $\blacksquare$  male 63 years-old VA 0.2) the changes were extensive. Data of normal controls from Sjostrand & Frisen (1977)

large borders - small field TV system

CSC was measured in different types of macular disease: senile macular degeneration, central serous retinopathy, fundus flavimaculatus, vitelliform macular degeneration, and retinal detachment including the macula. The macula is morphologically and functionally non-uniform and since macular disorders may involve the foveal, perifoveal and perimacular region or the receptor or neuronal cells

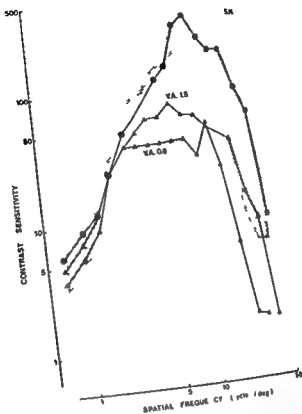


Fig 3  
CSC (1.4) in central serous retinopathy Case S.H. (male 49 years old) with involvement of right eye (Δ lower curve was obtained during the acute phase V A 0.6 and the upper curve following recovery V A 1.5) The CSC of the contralateral left eye (○ A. 1.5) is indicated by filled circles (●) Dotted area shows the range of seven normal controls aged 3 to 63 years

differently variation in effects on the contrast sensitivity function may be anticipated and is in fact demonstrable in this study

**Senile macular degeneration** In senile macular degeneration pathological changes occur within the macular area (Sarks 1976) giving rise to scotomata of varying size in central vision

The CSC was impaired in senile macular degeneration and the decrease was notable at high and intermediate frequencies in cases with reductions in visual acuity to 0.5-0.7 (Fig 2 case I A V A 0.7) In advanced cases of degeneration abnormality of CSC across the whole spatial frequency range was observed as illustrated by case L.L. (V A 0.2 in Fig 9)



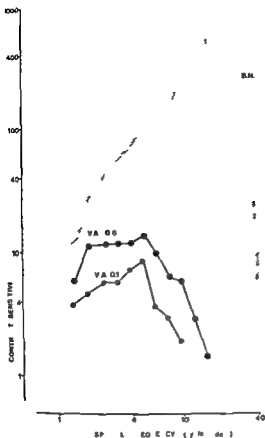


Fig. 4

B V female 24-years-old CSC (1.4°) of the left eye (●) of this patient with *fundus maculatus* with macular involvement (Stargardt's disease). The upper curve was obtained 26 (VA 0.6) and the lower curve after a period of progressive deterioration of central vision 2½ years later (VA 0.1). The dotted area represents the range of five normal subjects aged 19 to 31 years (from Frisén & Sjöstrand 1978).

In 2 patients with macular oedema at the time of the initial test, a subjective improvement and also an improved CSC was demonstrated at a second determination 2-6 months following the initial test.

A rough correlation was seen between the contrast sensitivity level between 3 and 6 cycles/degree and the visual acuity in patients selected to be in a stationary phase of disease with no obvious retinal oedema (results not shown).

**Central serous retinopathy.** In this macular disorder macular oedema develops due to leakage through the pigment epithelium. In most cases tested a detachment of the

pigment epithelium in the macular region (Gass 1967) was observed. During the acute phase the patients complained about impaired discrimination of fine details, metamorphopsias and micropsia (Frisen & Frisen 1979).

CSC was followed during the course of the disease and during the recovery phase contrast sensitivity was decreased for high and intermediate frequencies. Over the years the CSC improved parallel to the increase of visual acuity. Fig 3 demonstrates changes in CSC in one case during acute and recovery phase. It is notable that during resolution when the visual acuity had improved to 1.5 the patient still complained about slight blurring of the vision and the CSC was still markedly abnormal in the high and mid frequency region (Fig 3).

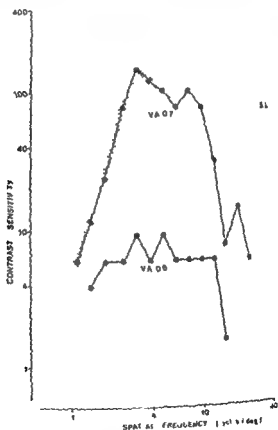


Fig 3

Case S 1, male 45 years old CSC (3.4) of the left eye (●) of this patient with macular degeneration with slowly progressive deficit in central vision. The upper curve obtained in 1976 (VA 0.7) and the lower curve (VA 0.6) 2½ years later. Data are within the range of seven normal controls aged 37 to 63 years.

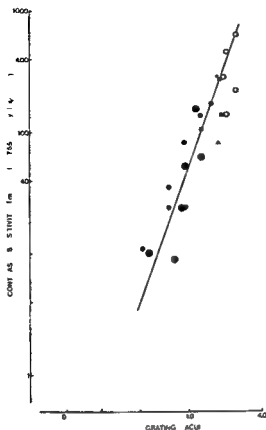


Fig 6

The relation between the mean contrast sensitivity in the mid frequency range (10-12 cycles/deg) obtained with the small field TV system (14) and grating acuity. Grating acuity was measured monocularly by means of sinusoidal gratings displayed on the TV screen at a distance of 7.9 m (for details see Andersson & Sjöstrand 1979). Fifteen cases with healed retinal detachment involving the macula were examined: ★ normal controls 19 to 42 years-old; ○ normal controls 43 to 70 years-old; ● macular detachments aged 19 to 41 years; ● macular detachments aged 42 to 70 years; ▲ fellow eyes for patients aged 19 to 41 years; ■ fellow eyes for patients aged 42 to 70 years.

**Various maculopathies** In this group various progressive maculopathies such as fundus flavimaculatus (Stargardt's disease) and vitelliform macular degeneration were investigated. During the time period of this study (3 years) the visual acuity dropped in several of the patients studied. Fig 4 demonstrates the CSC-curve of a 4-year-old female with fundus flavimaculatus involving the macular region.

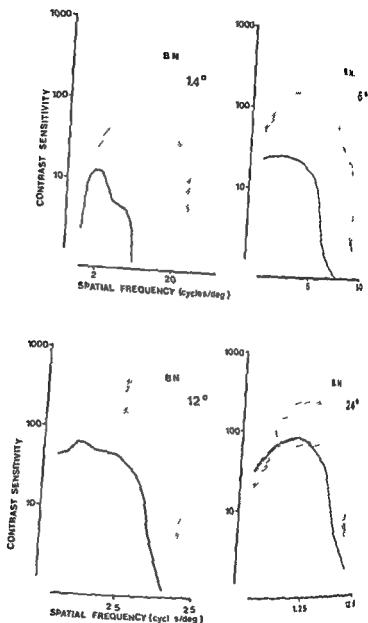


Fig 7

Case B V with fundus flat macula (the same patient as described in fig 4) CSCd at eye (solid line V 1.4°) of this patient using the large field TV system (6-94). The dashed area shows the range for 5 (6°) and 6 (12° and 24°) normal controls. To investigate the CSC at an angle of 1.4° at the eye (the same angle as that used with the small field TV system) the TV system ordinarily used for large field the screen was masked to 1.4° with a cardboard mask with the same background luminance as the surroundings. Dashed area in the 1.4° field indicates the range for six normal controls a) 1.4° and 6° b) 1.4° and 12°

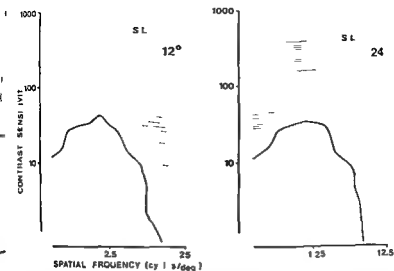


Fig 8

SL with vitelliform macular degeneration (the same patient as described in Fig 3) CSC of left eye (solid line V A 0.6) with the large field TV system (12° and 24°). The CSC for 6° is even more severely depressed (results not shown). Dotted area shows the range for six normal subjects.

ready 3 years ago when the visual acuity was 0.6 the CSC curve was impaired. When studied half a year ago both the visual acuity (0.1) and the CSC had dropped further (Fig 4).

Another patient with vitelliform degeneration of the macula studied during the same time period is shown as comparison. During the time period of study visual acuity only decreased from 0.7 to 0.6 whereas the CSC depression was markedly augmented over the whole frequency range (Fig 5). At the same time the patient experienced increasingly severe metamorphopsies and the macular status deteriorated as visualized by the ophthalmoscope.

**Retinal detachment including the macula.** We have investigated a number of patients with rhegmatogenous retinal detachment including the macula and followed the retinal function with various tests postoperatively (Andersson & Sjostrand 1979). It is known that age and refraction of the patient as well as duration of macular detachment preoperatively are of great importance for the functional outcome (Mundy & Davies 1974; Kreissig 1977).

Following a successful operation of macular detachment an initial rapid increase in visual acuity is found followed by a second phase of prolonged visual improvement extending over more than a year (Kreissig 1977). The spatial processing in the

reattached macula was followed by measuring the contrast sensitivity function and grating acuity during the recovery phase.

In a group of patients examined about a year after the operation an elevated CSC could be observed in the two age groups studied (Anderson & Sjostrand 1979). The high and intermediate frequency ranges were affected predominantly. Within the low frequency range the contrast sensitivity essentially was within the normal range. The reduction of the contrast sensitivity in the mid frequency range was roughly correlated to the visual acuity and grating acuity in these patients.

#### Macular disorders—large field TV system

To obtain a better insight into the field related visual problems of patients with visual disorders a few of the patients measured with the small field TV system were selected for further study using a large field TV system with a visual angle of 60° subtended at the eye.

The functional area for summation to threshold for sinusoidal gratings is a function of both the length of the bars and the number of bars of the grating (Howell & Hess 1979). By using a field of 24° the length of the bars and the number of cycles are above the critical limit at 0.5 cycles/degree giving rise to a valid contrast sensitivity function at this spatial frequency and above it. With the smaller field size an artefactual depression of the low spatial frequency end of the contrast sensitivity function is introduced (Estevez & Cavonius 1976; Howell & Hess 1979). However in more localized macular lesions a small field size is important in order to assess contrast sensitivity changes in fovea near regions of the macula.

An illustrative case is the 24-year-old woman (B.V. cf. Fig. 4) with bilateral flavimaculatus with a severe macular involvement giving her a visual acuity of 0.1 at the time of test with the large field TV system. As demonstrated by Fig. 1 a marked depression of the whole CSC curve is found for a field size of 14° and 6° with the small TV system. At an angle of 24° subtended at the eye the CSC curve is almost normalized at lower spatial frequencies demonstrating the striking field dependence that is found in some of the maculopathies studied. In the case with macular degeneration (case S.L.) however the CSC depression at lower spatial frequencies was observed also with the largest screen size used (Fig. 8) indicating more extended lesions in the posterior pole of the eye.

#### Discussion

During the last decade psychophysical and evoked response methods have been used to measure the capacity of the human visual system to observe certain patterns (Campbell & Maffei 1970; Campbell 1974; Sekular 1974). Spatial

otic gratings of variable contrast have been used as targets to study the ability to resolve low-contrast patterns under various experimental and clinical conditions as has been suggested that there may be in the visual system channels tuned to narrow ranges of spatial frequencies i.e. neurons exist in the visual system which are selectively sensitive to certain sizes of visual targets (Blakemore & Campbell 1969; Campbell & Maffei 1970; Campbell 1974). Our knowledge of the contrast sensitivity in disease, however, is more limited (for discussion see Arden 1978).

The results reported here have demonstrated that measurement of contrast is not only useful for describing visual loss for objects larger than the resolution limit but also of value for tracking progression or recovery in agreement with a preliminary study (Sjostrand & Frisen 1977). The slope of the line in Fig. 11 relating contrast sensitivity to grating acuity in postoperative cases of macular detachment indicates that the contrast sensitivity measurement is as sensitive as grating or any other acuity.

In early stages of maculopathies mainly the contrast sensitivity of the high- and middle frequency range was attenuated using the *small field TV system*. In advanced stages of maculopathies the pattern of contrast sensitivity depression was similar to that in acute cases of optic nerve disease (Frisen & Sjostrand 1978) and anisometropic amblyopia (Sjostrand 1979) with impairment over a wide range of spatial frequencies.

In spite of the disadvantages of using the *small TV screen* this system assessed contrast sensitivity function of the central spatial vision without interference of more peripheral vision. It has been shown that contrast sensitivity along a vertical line through the fixation point falls off steadily from a maximum at the centre (Robson & Graham 1979). However, this decline with eccentricity is most marked at high spatial frequencies and is small at lower spatial frequencies. The results with an impairment over the whole spatial frequency range using the *small field system* presumably has an involvement of the central area subtending  $1.4^\circ$ . Selective impairment of high spatial frequencies indicate that a peripheral part of the  $1.4^\circ$  stimulation field is spared.

Using the *large field TV system* with a field size ranging from  $6^\circ$  to  $24^\circ$  it was possible to study the contrast sensitivity when retinal summation was allowed over a larger field. A strong field-dependence of the CSC-deficit was found in many of the maculopathies restricted to the central part of macula. In such cases the contrast sensitivity function at lower spatial frequencies was almost normal with grating subtending an angle of  $24^\circ$  at the eye (Fig. 7). In cases with extensive lesions in the posterior pole a subnormal CSC over the whole frequency range was still seen with a large stimulation field. Similar findings have been found in the acute phase of optic neuritis. The CSC impairment over the whole frequency range appears to be compatible with a disturbance of a majority of the afferent visual channels that

subserve central vision. The differential field-dependence provides some insight into daily visual problems of patients with macular disorders. Hess et al (1976) have investigated a case with a unocular central 6° scotoma and they conclude that the macular region contributes significantly to the visibility of objects as large as 2.5° by 1.5°. A marked impairment of the low spatial frequency end of the contrast sensitivity curve however indicates involvement of a much larger area (cf. Robson & Frisvold 1979). The present study emphasizes that it is important to test CSC at different discrete regions over the visual field to assess regional differences.

Previous studies of contrast sensitivity with stimuli of sinusoidal gratings displayed on a TV- or oscilloscope screen have described abnormalities in contrast detection in patients with myopia (Fiorentini & Maffei 1976), amblyopia (Freeman & Thibos 1976; Levi & Harwerth 1977; Hess & Howell 1978) and supranuclear lesions of the visual pathways (Bodis Wollner 1972, 1974, 1976). In a recent study using a book of printed gratings, Arden & Jacobson (1978) have demonstrated subnormal contrast sensitivity in glaucomatous patients.

The majority of these studies give no evidence for a selective vulnerability of various spatial frequency ranges. The pattern of CSC attenuation is generally similar for a number of clinical disorders with mainly high frequency loss in disease such as corneal oedema (Hess & Garner 1977), strabismic amblyopia (Sjostrand 1969), retinitis pigmentosa (Wolkstein et al 1977) and early maculopathy (this study) and more general depression of CSC in optic nerve disease (Freeman & Sjostrand 1978), anisometropia (Sjostrand 1979) and advanced maculopathy (this study).

In conclusion, our study has shown that measurements of spatial contrast sensitivity in visual disorders supplements traditional visual acuity measurements and is useful for tracking progression or recovery of macular disease. Based on the present findings, a less time-consuming test might be constructed using the selected spatial frequencies at high, intermediate and low frequencies. However, the definite role of CSC for diagnosing or distinguishing different clinical disorders is lacking. The possibilities to use contrast measurements other than at threshold should therefore be explored. In our laboratory we are currently investigating the effect of disease on the supraliminal contrast level.

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# MORPHOLOGICAL HISTOCHEMICAL AND X RAY MICROANALYTICAL EXAMINATION OF DEPOSITS ON SOFT CONTACT LENSES IN EXTENDED WEARING

BY

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The deposits on 99 contact lenses of various water content from 90 wearers were analysed by the methods given in the title Six lenses were used as bandage lenses the remaining for optical correction The age of the patients varied from 7 to 56 years two-thirds being under 40 years

Wearing time had been from 1 week to 1 year with an average of 19 weeks The results obtained by the methods applied showed that calcium was present in just over two-thirds of the cases (90/99) other elements being infrequent Mucopolysaccharides were found in just under two-thirds (18/99) Chlorine was present in one fifth of the cases No significant amounts of lipid were detected Fungi were found in three cases (3/99) Bacteria were also found in these cases but never without fungi

Evaluation of the methods applied showed that the methods of choice were macroscopical examination and scanning electron microscopy in combination with X ray microanalysis in a few cases combined with histochemistry None of the methods applied is sufficient for protein analysis

*Keywords:* soft contact lenses - extended wearing - deposits - histochemistry - electron microscopy - X ray microanalysis

Deposits on soft contact lenses (CI) are an ever increasing source of unsuccessful lens wear. Knowledge of the nature of deposits is a mandatory prerequisite for the recommendation of effective cleansing methods with the object of prolonging the life of lenses.

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Table I

Previous examination of deposits

Year	Author(s)	Number	Type of lens
1972	Matas et al	5	Various
1973	Tragakis et al	50	Hema, PVP
1973	Tripathi & Ruben	1	Bionite
1975	Doughman et al	19	B-L soft lens Bausch & Lomb
1975	Lowther	2	
1975	Ruben et al	15	Various**
1976	Hilbert et al	2	B-L soft lens
1977	Cochet	969	Various**
1977	Kanai et al	18	
1977	Kintworth et al	9	Softicon
1977	Wedler	10	B-L soft lens
1978	Allen et al	6	Sauflon 85

\* Griffin Kontur Bausch &amp; Lomb

\*\* Sauflon Bionite Poly Hema, PVPMA

\*\*\* Unspecified

(n) Indicates n lenses examined by the method

Thorough examination of the deposits applying various physical chemical and morphological methods has been carried out on a somewhat limited number of lenses (Table I)

The present study was planned to include a combination of light microscopy, histochemistry (LM), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and X ray microanalysis (X ray) of deposits on contact lenses. Various water content worn for extended periods to ascertain the composition of these deposits and to evaluate which methods were appropriate for routine use.

### Material and Methods

A total of 29 hydrophilic lenses from 20 patients was examined. Table II lists the ages of patients with a range from 7 to 86 years, two-thirds being under 45 years of age. Most of the patients wore CL because of myopia, only five because of corneal or conjunctival disorders. The continuous wearing period had varied from 1 week to 1 year with an average of 13 weeks. All lenses had been discontinued because of troublesome and unremitable symptoms.

Table 1 (cont.)

fit contact lenses

Methods of examination										Results
r	Bioch	Cult	LM Histo- chem	SEM	TEM	X ray micro	X ray dif	Atom Abs	Spect phot	
				+						Bact
		+	+							Bact
					+					Debris Bact <sup>2</sup>
	+(6)		+(3)	+(8)	+(2)					Lip
			+				+			Pr MPS Ca
	+		+	+	+	+	+(2)	+	+	Ca <sub>2</sub> (Pos) <sub>2</sub> Si S Cl
			+							Pr MPS Ca
					+	+				Pr Fu
	+		+			+				Ca Fu
	+					+				Ca Pr
										Pr MPS Lip
			+(1)					+(1)		MPS Lip Ca

examinations

light microscopy SEM scanning electron microscopy

TEM transmission electron microscopy Pr protein MPS mucopolysaccharides

lipids Fu fungi

Two-thirds of the lenses were of the SCANLENS 4th type a PVP/PMMA (polyvinylpyrrolidone/poly methyl methacrylate) with a water content of 16%. The lenses were stored at 4°C until use. Upon examination each lens was photographed in situ and in sectors in a photomicroscope (Wild M 400) using light field and dark field illumination. All lenses were processed according to routine paraffin technique. The last half of the series was sectioned and appropriate parts were also processed for LM as well as for TEM and TEM.

Initially lenses were fixed in 4% phosphate buffered neutral formaldehyde for 24 h at 4°C, dehydrated in ethanol and paraffin embedded. In some cases half a lens was cut in a half (Am. Opt. Corp.) and stained with Luxol fast blue (Luxol) Sudan black B (black B) and oil red O (red O). Deparaffinized sections were stained with haematoxylin-eosin (HE) haematoxylin-phloxine-safranin (HPS) periodic acid-Schiff (PAS) Alcian blue with 0.1 M and 0.1 M HgCl<sub>2</sub> (AB) alizarin red S (Aliz) the von Kossa method (Kossa) murexide staining a murexide method (Mur) silver nitrate-rubeanic acid a murexide method (Rub) the oxidized murexide method (OTA) Grocott's modification of Gomori's methenamine silver nitrate technique (Grocott) Gridley's method for fungi (Gridley) and Brown-Bren's bacterial stain (B).

For SEM critical point dried (acetone CO<sub>2</sub>) air dried or formalin vapour exposed specimens were used. Specimens without or having a sputter coating of Au/Pa of 300–400 Å.

Table II  
Age of patients, diagnosis, wearing time and type of lens

Patient No	Lab	Sex	Age	Diagnosis	Wearing time (weeks)	Type of lens
1	415/77	F	61	Myopia		Plano-T
2	457/77	M	70	Pemphigus -	II	Plano-T
3	458/77	-	-	Conj	-	-
4	485/77a b	F	40	Myopia	4	Scalera *
4	486/77a b	F	28	Myopia	4	Scalera *
5	487/77a b	M	20	Myopia	9	Scalera *
6	488/77	M	29	Myopia	9	Scalera *
7	495/77	M	15	Keratitis -	1	Scalera *
7	517/77	-	-	neuroparalytica	1	-
9	706/77	F	86	Keratitis bullosa		Plano-T
11	778/77	M	19	Aphakia	24	Wexia
10	1198/77	M	27	Myopia	59	Wexia
11	1238/77	M	7	Anisometropia		Scalera *
12	1244/77	M	76	Keratitis bullosa	90	Wexia
13	99/78	M	71	Aphakia	15	Scalera *
14	116/78a b	M	26	Myopia	9	Scalera *
15	163/78a b c d	F	90	Myopia	5	Scalera *
16	230/78					Scalera *
17	251/78	F	21	Myopia	II	Scalera *
18	266/78	M	30	Myopia	98	Scalera *
19	307/78	F	42	Myopia	7	Scalera *
20	351/78	M	41	Myopia	21	Scalera *

a b Means a pair of lenses

a b c d Mean two pair of lenses worn for the same period

In cases 2 and 7 the two lenses indicated in the table are from the same eye. No lens of patient No. 16 were available

thickness were examined. The SEM specimens were used for elemental analysis. A total of 4 lenses was scanned and microanalysed. For TEM 1 mm<sup>2</sup> parts with deposits were cut out of the lens, fixed in 4% cacodylate buffered glutaraldehyde at room temperature for 1 h, post fixed in 2% OsO<sub>4</sub> for 2 h, dehydrated in graded acetone and embedded in a resin. Survey sections of 1 µm and ultrafine sections were cut with glass knives on to diamond knife in a Reichert ultramicrotome OM U3, the survey sections being stained with lead citrate blue and the fine sections with 1% uranyl acetate for 45 min and 1% lead citrate for 15 min. Five lenses were used for TEM.

SEM was performed in the Jeol JSM 35 at 15, 10 and 7 kV and TEM in either the Zeiss EM 9 S-2 at 60 kV or the JEOL 100 C at 60-80 kV. Elemental analysis was carried out in the JEOL analytical Temscan 100C/100CX at 40 kV.

A survey of the technical procedures is given in Table III.

Table III  
Technical procedures

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*Macroscopical examination*

- a Examination in dissecting microscope
- Macrophotography
  - 1 light field
  - 2 dark field

*Histopathologic examination*

- a Paraffin sections
- b Frozen sections
- c Staining
  - HE HPS PAS AB Aliz Kossa Mur Rub OTA
  - Crocott Gridley II B Luxol black II red O

*SEM*

- a Air dried
  - b Formalin vapour exposed
  - Critical point dried
- } coated  
 } /  
 } uncoated

*X ray — microanalysis*

Performed on SEM specimens

*TEM*

Glutaraldehyde — araldite/epon

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## Results

Table IV is a summary of the methods applied in the individual cases with a survey of the composition of the deposits as interpreted from the study. Macroscopical examination (Fig. 1) and light microscopical histochemistry were performed in all cases. TEM in five and SEM and X ray microanalysis in 14 cases. Initially when LM was exclusively used we interpreted a positive AB alone or together with a positive AS as manifestation of MPS but not a positive PAS alone. To accept the presence of calcium using LM alone at least two of four methods for Ca (Aliz, Kossa, Mur, Rub) should be positive. Sulphate-containing MPS were considered present when spectrum containing a peak for S was found together with positive histochemical stainings (Fig. 2). Although the histochemical stainings were negative MPS might be the source of the sulphur in the event of an S peak. However we avoided such a recording. Prominent Cl peaks were found in nine cases together with traces of

Table II  
Survey of methods and results

Patient No	Lens No	LM	TEM No	SEM No	$\lambda$ ray	Type of biopsy
1	1	+				
2	2	+				Ca, NPS
2	3	+				Ca, NPS
3	4	+				Ca, NPS
3	5	+				Ca, NPS
4	6	+				Ca, NPS
4	7	+				Ca, NPS
5	8	+				Ca, NPS
5	9	+				Ca, P, NPS
6	10	+				Ca, P, NPS
7	11	+				Ca, NPS
7	12	+				Ca, P
8	13	+				Ca
9	14	+				Ca
10	15	+				NPS
11	16	+	251	251	+	NPS, Fe, Cu
12	17	+	253		+	NPS, Fe, Cu
13	18	+		263	+	Ca, NPS
14	19	+		264	+	P
14	20	+	269	269	+	Ca, Mg, Cl, Fe
15	21	+		271A	+	Na, Cl
15	22	+		271B	+	Ca, Na, Cl
15	23	+		275	+	Na, Cl, Fe, NPS
15	24	+	276	276	+	Na, Cl, S
16	25	+		280	+	Ca, Na, Cl, Fe
17	26	+	281	281	+	Ca
18	27	+		286	+	Ca, Na, Cl, NPS
19	28	+		299	+	Ca, Cl, NPS
20	29	+		295	+	Ca, Cl, S, NPS
Total examinations	29	29	7	14	14	

Abbreviations: see Table I

other elements and in six cases together with sodium. In the latter cases the Na-X may be sodium chloride from tear fluid, storage fluid or lens material. Staining for protein were only positive in two cases. One of these cases was also analysed by  $\lambda$  ray microanalysis without definite peaks. This was expected, since light elements such as carbon, oxygen and nitrogen, the main elements of proteins, cannot be significantly detected by electron beam  $\lambda$  ray microanalysis. The same applies to lipids. Lipids in significant amounts were not detected in the frozen sections.



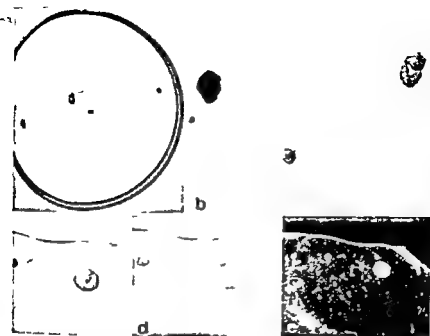


Fig 1

Microscopical view of smooth globular deposits in light field and dark field.

Whole lens (Lab No 116b/8  $\times 4$ )

Detail of a. ( $\times 12$ )

Deposit in transmitted light (Lab No 1244 ( $\times 30$ ))

Same in transmitted tangential light ( $\times 30$ )

Same in dark field illumination ( $\times 20$ )

Following the above principles the analyses showed that calcium was present in possibly 21 out of 29 lenses (Fig 3) although in varying amounts (Table IV) as was to be expected as the composition of the deposits varied on the same lens. It is noticeable that calcium and fungi do not particularly go together (Table IV) and never bacteria were present in the three cases of fungal contamination as if the two organisms were in symbiosis (Fig 4). It is impossible to judge from morphology by light microscopical or electron microscopical methods or by staining methods the exact kind of fungi but probably *Aspergillus* was involved in at least one case. The bacteria had the shape of small rods. MPS were found in 18 out of 29 lenses. However three of these cases were less significant because of insufficient material for analysis. Only biochemical analysis can identify any linkage with protein. Light microscopically and in SEM the most common type of deposit was round and smooth (Figs 1 and 3). More rarely it had a crystalline appearance (Fig 2).

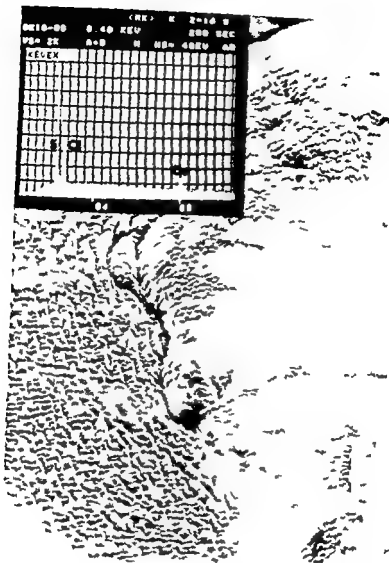


Fig 2

Crystal like deposit containing MPS. The energy dispersive spectrum at upper left corner shows peaks at 2.3 keV ( $\lambda_{K\alpha 1}$  for S) and 2.6 keV ( $\lambda_{K\alpha 1}$  for Cl). The sulphur peak arises from the sulphated MPS and the chlorine from metal salts. The peak at 8.0 keV ( $\lambda_{K\alpha}$  for Cu) is due to copper in the specimen holder (Lab No 32078, SEM 700).



*Fig 3*  
A large globular deposit containing Ca and sulphated MPS. The  $K\beta$  (3.7 keV) and the  $K\alpha$  (4.0 keV) peaks of Ca are prominent. The atomic marker is placed above the S-peak.  
(Lab No 302/78 SEM 2.0k)



Fig. 4

Fungal hyphae (large arrow) covered with small rod like bacteria (small arrow). The energy dispersive spectrum depicts phosphorus and sulphur from organic matter. In addition calcium was present (Lab No 1938/77 SEM 251)



Fig 5

Macroscopic view of fungi illustrating the easily detected colonies  
(Lab No 1238/77 ( $\times 30$ ))

## Discussion

Exogenous contamination of contact lenses must be derived from substances dissolved in the precorneal film. Small molecules able to penetrate the pores of the lens polymer will only precipitate when insoluble while macromolecules (proteins, polysaccharides and lipids or compounds of these) tend to cover the surface of the lens. Insoluble calcium salts were previously considered to be the main source of contamination. Later protein coatings were regarded as the overwhelming problem and much effort was spent in developing proteolytic enzymes to remove this impurity.

In our material mucopolysaccharides (MPS) were one of the main components in the deposits. MPS have also been found in a few of the previous investigations (Allen et al 1978, Hilbert et al 1976, Lowther 1975, Wedler 1977). MPS in combination with calcium (Ca) and less frequently with other elements form the common smooth spherical type of deposit. In the few cases where MPS were the only constituent of the deposit a microcrystalline appearance was observed by microscopy in high magnification and particularly in SEM.

Using the less sensitive histological methods Lowther (1975) observed a crystal-like type of deposit containing calcium on lenses for day time use but only in lenses accidentally boiled in saline prepared from calcium rich artesian water. However

he failed to detect calcium in the common smooth deposits on le wear pr 4  
boiled

On one lens Allen et al (1978) found neutral MPS in smooth deposits with Ca, protein and lipids in a multilayered arrangement. We have not observed such a structure on our frozen sections stained for lipids, as no significant amount was present.

Doughman et al (1975) detected exclusively lipids and lipoproteins in deposits on their lenses.

The above mentioned differences in content of substances in the deposits and the difference in type of deposit may be explained partly by the different methods used and partly by the differences of lens material. Individual methods of wear tests also be of importance.

The assumption that proteins are a main component in lens deposits is primarily based on biochemical investigations (Wedler 1977). Using the far less sensitive histological techniques we could only detect protein in two out of 99 lenses.

Our examination revealed that more than two-thirds of lenses worn for extended periods contained calcium. According to the literature the calcium content however seems to vary, and calcium can even be demonstrated in stock lenses (Allen et al 1978). Ruben et al (1975) demonstrated that Ca is deposited as calcium triphosphate. This observation was supported by Minnworth et al (1977) who found only Ca together with phosphorus (P) in one out of eight lenses.

Ca together with fungal deposits was found only in one out of three lenses. It was found in most of the deposits; this rare combination of fungi and Ca may indicate that fungal contamination of Ca-containing deposits is not the rule.

Fungal deposits are easily detected macroscopically as dark colonies (Barnes et al 1974; Cochet 1977; Kanai et al 1977) (Fig. 5). In our material bacteria were a heavy contaminant when fungi were found. The bacteria were always in contact with the hyphae of fungi, suggesting a form of symbiosis. This was confirmed by the fact that our study could not demonstrate any bacteria in the absence of fungi. Previous studies (Doughman et al 1975; Kanai et al 1977; Minnworth et al 1977) also demonstrated only a few bacteria on soft contact lenses.

Evaluating the methods applied, we would recommend macroscopical examination combined with low voltage SEM of formalin vapour fixed non-coated sections of lens and X-ray microanalysis of the same specimen. In a few cases supported by IM, TEM may give some structural information, particularly in the case of bacterial deposits but the information achieved can be easier reached by the other methods.

The present work indicates that the recommended methods give a useful description of the composition of the deposits that MPS and Ca are dominant parts of the deposits and that bacteria and fungi are rare and when present are always found together.

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# OPTICAL PRINCIPLES FOR ESTIMATION OF ENDOTHELIAL CELL DENSITY WITH THE NON CONTACT SPECULAR MICROSCOPE

BY

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In non contact specular microscopy of corneal endothelium endothelial cell density is overestimated due to the angle of observation and the curvature of cornea. On the basis of theoretical considerations it is concluded that if the angle of observation is kept at a small value the curvature of cornea contributes only insignificantly to this overestimation the major determinant being the angle of observation. When an angle of  $46^\circ$  is chosen between slit illumination and optical axis of the microscope it is calculated that estimates of endothelial cell should be multiplied with a factor of 0.929 to correct for the angle effect. Six eyes were photographed with both a contact and non-contact specular microscope and endothelial cell density estimated. Mean observed difference in cell counts was +5.3% without correction and +1.9% with correction for angle effect. 95% confidence limits for the difference with correction are -3.7 and +6.0% respectively showing that estimates of endothelial cell density obtained with the non contact specular microscope agree closely with those obtained by contact specular microscopy when corrected for angle of observation.

**Key words:** endothelium - cell density - specular microscopy - cornea

Specular microscopy of corneal endothelium *in vivo* is generally performed with the technique described by Maurice (1968). This technique implies application of the cornea with a dipping cone mounted on the objective (Laing et al. 1973; Boxer et al. Kaufman 1976). The appplanation is of help in keeping the endothelium flat and allowing a high magnification (about  $\times 200$ ).

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The technique of visualizing the endothelium with the slit lamp was originally described by Vogt (1920). However, it is not until recently, after suggestions made by Olle Holm, University of Lund, Sweden, that this non-contact technique has been reintroduced into clinical practice combined with a photographing device (in 1978).

The non-contact specular microscope is in principle an ordinary slit lamp mounted with a microscope of high magnification (about  $\times 100$ ). The objective of the microscope has a long working distance which allows the endothelium to be viewed without contact with the anterior corneal surface.

The reflex from the endothelium can be dissociated from the strong reflex from the corneal surface by increasing the angle of observation. In contact specular microscopy the microscope objective also serves to focus the incident slit illumination on the cornea. The angle between incident light and reflected light is with this technique very small. In non-contact specular microscopy the angle is larger and it significantly disturbs the dimension of the photomicrograph so that the area covered on the photograph corresponds to a greater corneal area. Endothelial cell density estimated from photographs taken with the non-contact specular microscope may therefore erroneously be too high if not corrected. Another factor which induces too high estimates of cell density is the curvature of the cornea.

The purpose of this study was to provide a theoretical basis for correction of estimates on endothelial cell density obtained with the non-contact specular microscope. To illustrate the order of magnitude by which these factors play a significant role, estimates obtained with non-contact specular microscopy are then compared with estimates of endothelial cell density obtained with the contact specular microscope *ad modum* Maurice.

## Methods

The non-contact microscope was produced by Preidler Instrument AB, Malmö, Sweden. The objective was a Leitz objective  $25\times$  magnification with a working distance of about 15 mm. An Olympus OM 1 camera body was mounted directly on the microscope. The microscope was mounted on a metal arm designed to fit a Zeiss slit lamp and adjusted so that the microscope could be circled around the slit point. The angle between the incident light from the slit lamp and the optical axis of the microscope could be kept constant (in the present study at  $46^\circ$ ) by mechanically fixing the slit lamp and the microscope to each other. Photographs were taken using the Zeiss flashing device for the slit lamp and Ilford PAN F 24 $\times$ 36 film, secondarily enlarged to 18 $\times$ 24 cm.

The contact specular microscope was purchased from Syber Inc., Canesville,

Florida Film Kodak TRI X 21×36 mm Secondary enlargement to 1/10  
Final magnification was determined by photographing a Burger T-1200 cell counting chamber

Six normal subjects (3 left and 3 right eyes) were photographed with a non contact specular microscope and the contact specular microscope. On the basis of greatest photographic clarity photographs were selected. Endothelial cell density was estimated from four or more pictures according to the counting principle given by Gundersen (Sperling & Gundersen 1979) which gives unbiased estimates of cell density to be drawn from small samples

95% confidence limits were calculated on basis of the t-distribution.

## Results

### Theoretical considerations

Light is always reflected from the endothelium at an angle which practically equals that of incident light. Due to refraction of cornea, however, the endothelium is observed at an angle ( $u$ ) which is smaller than the angle of incident light on the cornea ( $i$ ) (Fig. 1). The observation angle  $u$  is determined by

$$\frac{\sin i}{\sin u} = n_c$$

where  $n_c$  denotes refractive index of cornea. Substituting  $i = 15^\circ$  (see Davila 1971)  $n_c = 1.37$  (the angle used in the present study) one gets  $u = 16^\circ$ . If the endothelium is

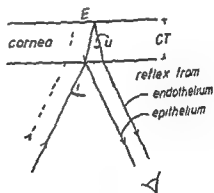


Fig. 1

In non-contact specular microscopy the endothelium is seen at an angle  $u$  which is smaller than the angle  $i$  of incident light on cornea.  $u$  is determined by  $\frac{\sin i}{\sin u} = n_c$  where  $n_c$  is refractive index of cornea. The maximum width  $W$  of endothelium observed is limited by the strong reflex from the epithelium and is determined by  $W = 2 \cdot CT \cdot \tan u$  where  $CT$  is corneal thickness (assuming parallel beams of light).

this angle of observation would induce a shortening of the horizontal dimension by a factor  $\cos \alpha$  assuming a horizontal angle between incident and reflected light. Since the vertical dimension would be unchanged this means that an area on the endothelium would be diminished by a factor  $\cos \alpha$ .

The curvature of cornea however further distorts the observed dimensions. In Fig. 2 is shown in the horizontal plane how the angle of observation and the curvature of cornea influence an observed area of endothelium. For simplicity the outer surface of cornea has been omitted. The narrow beam of slit light is reflected with maximal intensity in point P. The angle of observation in this point is  $u$ . In point Q the endothelium is also seen but more dimly because the angle of observation is slightly greater than the angle of incident light (the fact that the endothelium is seen as a band and not as a line is due to the slight non-parallelism of the slitlight and the non-smooth surface of the endothelium).

PQ denotes curved true distance on cornea the observed distance PR is determined by

$$\sqrt{(r^2)(\sin^2 \alpha + (1 - \cos \alpha)^2)} \cos^{1/2} \alpha + u \quad (1)$$

where  $r$  is radius of cornea  $\alpha = PQ$  in radians and  $u$  is angle of observation. The length of PQ seen on endothelium cannot be increased beyond the limit given by the distance from the anterior surface of the cornea due to the very intense illumination from the surface. See Fig. 1. The maximal length  $E$  is determined by

$$CT = r \tan u \quad (2)$$

where  $CT$  is thickness of cornea.

Assuming  $CT = 0.5$  mm and  $u = 16.5^\circ$  one gets  $E \sim 0.3$  mm. This corresponds to 0.038 mm on cornea ( $\alpha$  in Fig. 2) assuming cornea radius to be 8 mm ( $r$  in Fig. 2). Substituting these values in equation 1 the observed length PR of PQ (true distance 0.3 mm) becomes

$$\sqrt{(8 \text{ mm})^2 (\sin^2 0.038 + (1 - \cos 0.038)^2)} \cos^{1/2} \alpha + u$$

$$0.999 \times \cos^{1/2} \alpha + u$$

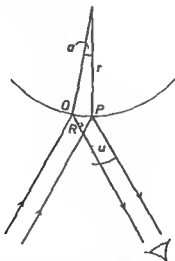


Fig. 2

In specular microscopy the observed distance PR corresponds to a distance PQ on cornea which is greater than PR depending on the angle of observation and the curvature of cornea.

$r$  = radius of cornea  $\alpha = PQ$  in radians

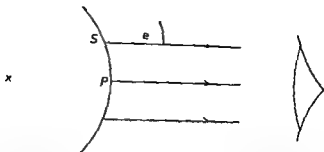


Fig 3

In non contact specular microscopy the curvature of cornea induces an observation angle  $e$  in the upper and lower part of endothelium observed when a vertical slit illuminates and

and this is almost

$$= PQ \times \cos(\frac{1}{2}\alpha + u)$$

Substituting  $u = 16.5^\circ$  one gets

$$PR = PQ \times 0.9533$$

If the cornea did not curve

$$PR = PQ \times 0.9588 (= PQ \times \cos u)$$

i.e. the curvature of cornea contributes to less than 1% in the shortening of the observed horizontal distance

With a vertical slit illumination the vertical part of the endothelium observed is greater than the horizontal part. With the present method the maximal vertical length of the endothelium observed is about 1 mm. As seen in Fig 3 because of the curvature of the cornea this induces an observation angle ( $e$  in Fig 3) which is greatest in the upper and lower part of endothelium photographed. If the cell density was counted in these parts, these areas would result in too high estimates. The overestimation is however insignificant.

If a small area in point S on Fig 3 is assumed to be 0.3 mm from P and Q and photographed this area would be observed at an angle of 0.34 radians (or 36.4 degrees). The length in S would consequently be shortened by a factor of  $\cos 36.4^\circ = 0.804$ , i.e. a insignificant error. This calculation assumes no refraction of the anterior surface of cornea. This refraction would of course tend to diminish the error even more.

These results show that if the endothelium is observed at a horizontal angle of observation of 16.5 degrees, incident light and observation the horizontal dimension is shortened by less than 1.4 per cent. The curvature of cornea contributes only insignificantly to this shortening. The vertical dimension is almost unchanged due to the insignificant curving of cornea in the middle part observed. It is therefore possible to correct an area observed with a refractive error, namely with the factor given in equation 4.

#### Non contact and contact specular microscopy of corneal endothelium

In Table 1 the results of estimates of endothelial cell density obtained with non contact as well as contact specular microscopy are given. The photographs taken with the non contact specular microscope (Fig. 4) are taken at an angle of 9 degrees of incident light. This corresponds to 16.5 degrees observation angle of endothelium (Fig. 3). It is

if no correction is made for the angle of observation the cell density is overestimated compared to the estimates obtained with the contact specular microscope. The cell densities obtained with the latter can be regarded as true ones due to the very small angle of observation. From the distortion on micrographs of the squared micro-scale of Burger Turk this angle was calculated to be less than  $6.1^\circ$  overestimating cell counts by less than 1 per cent.

The correction factor outlined in equation 4 ( $= 0.959$ ) is applied on the estimates obtained with the non-contact specular microscope. The mean difference in cell counts diminishes from  $+33$  to  $+12\%$  making the estimates almost ideal. 95% confidence limits for the mean difference with correction are  $-3.7$  to  $+6.0\%$  respectively.

### Conclusion

This study has shown that if not corrected endothelial cell density is overestimated by the non-contact specular microscope. The major determinant for this overestimation is the angle of observation while the curvature of cornea contributes

Table I  
Estimates of endothelial cell density (cells  $\text{mm}^2$ ) obtained with contact and non-contact specular microscopy (sp/m) with and without correction for angle of observation (mean  $\pm$  SEM)

Experiment	Contact sp/m	Non-contact sp/m		Difference (%)	
		Without correction	With correction ( $\times 0.959$ )	Without correction	With correction
TB	$2863 \pm 69.3$ (n = 9)	$3031 \pm 37.1$ (n = 5)	$2907 \pm 35.6$	+ 3.9	+ 1.5
JC	$2191 \pm 79.7$ (n = 12)	$2341 \pm 43.1$ (n = 7)	$2245 \pm 41.2$	+ 6.8	+ 2.5
HA	$2887 \pm 89.9$ (n = 11)	$3113 \pm 27.8$ (n = 6)	$2986 \pm 26.5$	+ 7.8	+ 3.4
U	$165 \pm 64.5$ (n = 6)	$1825 \pm 55.9$ (n = 8)	$1750 \pm 53.4$	+ 3.4	- 0.8
OH	$3390 \pm 84.5$ (n = 4)	$3529 \pm 49.7$ (n = 9)	$3384 \pm 47.7$	+ 6.3	+ 1.9
AJ	$2110 \pm 59.1$ (n = 10)	$2846 \pm 69.3$ (n = 5)	$2739 \pm 66.3$	+ 2.9	- 1.4

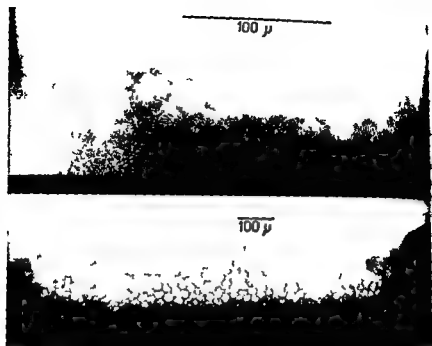


Fig 4

Normal corneal endothelium photographed in vivo with a contact and a non-contact microscope. The largest field of observation is seen with the non-contact specular microscope.

only insignificantly and may be neglected if the angle of observation is kept at a small value (in the present study at  $29^\circ$ ). When corrected for this angle effect, the estimates agree closely with those obtained by contact specular microscopy.

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# EFFECTS OF CORNEAL PRESERVATION DONOR AGE CADAVER TIME AND POSTOPERATIVE PERIOD ON THE GRAFT ENDOTHELIUM

*A specular microscopic study*

BY

PEKKA RUUSUVAARA

The endothelia of 102 clear human corneal grafts in 93 patients were photographed *in vivo* with a specular microscope. The endothelial cell densities of the grafts were counted from an area of 0.01 mm<sup>2</sup>. The follow up period averaged three years. The patients' ages ranged from 16 to 87 years and that of the donors from 8 to 89 years. The average endothelial cell density of all 102 transplants was  $1169 \pm 478$  cells/mm<sup>2</sup>.

Corneal transplants stored in Mh medium (N = 19) have more cells than cryopreserved grafts (N = 42) or grafts stored in the moist chamber (N = 41); this last group was used as control material. The differences in cell densities were statistically significant. The cell density of cryopreserved grafts was significantly higher than in grafts stored in the moist chamber.

Only in those grafts in which moist chamber storage had been used was endothelial cell density dependent on time of storage. With an increase in cadaver time (time from death to enucleation) the endothelial cell density of the graft decreased.

An inverse correlation was found between the cell density of the graft and donor age. Good results were still found with healthy donors over 60 years. The endothelial cell density of the grafts showed a steady decrease during the long term post-operative period, the rate of cell loss being much higher in graft endothelium than in normal endothelium during ageing.

*Key words:* cornea, corneal graft endothelium, corneal preservation - specular microscopy - cell viability - donor corneal material - cryobiology.



age of donor corneas has always been a major problem in corneal grafting (Miles 1939). Filatov started the use of cadaver eyes in the 1930s. Since that time preservation of the cadaver eye or the cornea alone has become increasingly important. Nowadays we need a well preserved cornea with potential function for a penetrating graft. A good criterion of adequate preservation of the cornea is intact functioning corneal endothelium (Breslin & Ng 1976).

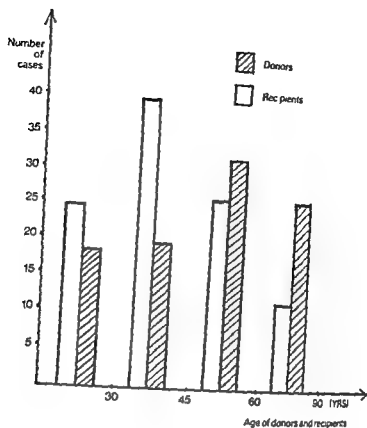
A successful corneal graft has to have optical clarity, regular curvature and normally functioning endothelium. The corneal endothelium functions firstly as a barrier and secondly as a metabolic dehydrating pump (Kaufman *et al.* 1966, Kaufman 1973). The success of the graft depends chiefly on whether the preserved cornea has a viable endothelium. The adult human endothelium is believed not to regenerate at all and when damaged the endothelial cells heal by spreading to cover the area of the dead cells (Bourne & Kaufman 1976b).

In 1968 Maurice described a specular microscope for studying the endothelium of intact cornea *in vivo*. This instrument has subsequently been modified by Gegg *et al.* (1975) and by Bourne & Kaufman (1976a). This *in vivo* photomicroscopy affords an exact method for measuring the density of endothelial cells after keratoplasty (Bourne & Enoch 1976, Sherrard 1976, 1977).

Studies made with the specular microscope have shown that corneal transplants have fewer central endothelial cells than normal corneas (Bron & Brown 1974, Bourne & Kaufman 1976b). The endothelial cell density of the graft does not appear to be significantly correlated with corneal thickness, rejection episodes, the prognosis of the recipient or the method of donor tissue preservation (Sato 1978). Older corneas transplanted from younger donors to younger recipients have a significantly greater number of central endothelial cells (Bourne & Kaufman 1976b). The results of previous studies on the significance of the different parameters of corneal grafting may give an unrepresentative picture because of the small numbers of patients and short follow-up periods.

The average endothelial cell densities in corneal grafts show a wide variation from one series to another (Bron & Brown 1974, Bourne & Kaufman 1976b). The highest density of endothelial cells was reported by Bourne *et al.* (1977) in *in vitro* cultured corneas with an average cell density of  $1548 \text{ cells mm}^{-2}$ .

Opinions still vary about the best methods for preservation of corneal buttons or globes and also about the critical preservation time and donor's age after which a cornea is no longer suitable for penetrating keratoplasty. With cryopreserved corneal tissue it has been calculated or recommended that the donors should be under the age of 50 years and the cornea must be excised within six hours of the death of the donor (Hung 1970). However, a fresh cornea or material used after moist chamber storage is still preferred to preserved material because the transplanted endothelium is assumed to have a better viability.



*Fig. 1*  
Age distribution of donors and recipients.

This paper reports the results of measurements of endothelial cell density in clear human corneal transplants made at the Helsinki University Eye Hospital. The first point studied was the extent to which the endothelial cell density of the graft depended on the method of preservation. In addition the importance of donor age, cadaver time (time from death to enucleation or excision of the cornea), preservation time and some other parameters were evaluated. The subsequent changes of the graft endothelium was also elucidated.

## Patients and Methods

### Patients

The clinical series comprised 102 penetrating keratoplasties in 93 patients. Of the graftings seven were bilateral and three were regraftings. This follow up study was made during the years 1976-78. The follow up period varied from 1 month to

s on average 3 years. The patients' ages ranged from 16 to 77 years and that of donors from 8 to 89 years (Fig. 1). Among the patients the ratio of men to women was 55 to 40.

#### *clinical diagnosis of the patients*

There were altogether 60 eyes with keratoconus and 8 patients had other corneal dystrophies. The corneal opacities were due to herpes, tuberculosis or lues in 7, 3, 9 cases respectively. In 11 patients the corneal inflammation had some other cause and in 4 patients the cause was trauma or unknown.

#### *operative methods and postoperative treatment*

Operations were performed mostly by the same two surgeons (SV and KH). The size of the graft varied from seven to eight mm. The graft was cut from the donor globe with Franceschetti's trephine or from the corneal button with Polack trephine (Brightbill et al. 1973).

During the last six years a microsurgical unit (Moller Wedel) has been used instead of the previous magnifying loupe. Before the operation the condition of the graft was checked with the microscope and after punching the graft the peripheral corneal rim was examined by vital staining of the endothelium.

As suture material a 10-0 nylon monofilament was used for continuous or single sutures. In the earliest cases 9-0 virgin silk sutures were used.

As prophylaxis all patients received topical or peroral corticosteroid and when the cornea was heavily vascularized the patient was also treated with immunosuppressives.

#### *methods of corneal preservation*

The patients were grouped according to the method of preservation of the donor corneas. The first group, which was used as control, comprised patients with grafts stored in a moist chamber as whole globes at 4°C for less than 48 h. Of these 16 were fresh, which means that the storage time was less than one hour. The total number of transplants in this group was 41. It is recommended that the time interval between enucleation and keratoplasty should be less than 48 h (Paton 1955).

The second group comprised patients who had a cornea after intermediate term preservation in the M-K medium devised by McCarey & Kaufman (1974). The donor cornea had been excised with a Draeger's electric rotor trephine within 11 h of death (S. Vannas 1975). In this method the preservation time varied from one to four days in tissue culture medium at 4°C. McCarey & Kaufman's recommendation for critical storage time with this method is up to five days.

The third group comprised grafts which had been cryopreserved according to

Table I  
Endothelial cell density and preservation methods ( $\pm$  s.e.)

Preservation method	Number of transplants	Mean age of donors (years)	Mean age of recipients (years)	Endothelial cell density (cells/mm <sup>2</sup> )
Cryopreservation	42	(N = 41) 51.4	40.0	1111 $\pm$ 13
Storage in M & K medium	19	(N = 19) 46.2	43.9	1045 $\pm$ 12
Moist chamber storage	41	(N = 46) 47.9	40.1	942 $\pm$ 12
Total	102	(N = 96) 49.1	40.8	1104 $\pm$ 11

Table II  
Endothelial cell density in transplants to patients with keratoconus and other diseases after different types of preservation

Preservation Method	Diagnosis of patients	Number of transplants	Mean age of donors (years)	Mean age of recipients (years)	Endothelial cell density (cells/mm <sup>2</sup> )
Cryopreservation	conus	27	(N = 26) 54.3	33.9	1111 $\pm$ 13
	others	15	46.3	39.3	1045 $\pm$ 12
Storage in M & K medium	conus	7	32.4	31.1	1420 $\pm$ 26
	others	12	39.4	51.8	1111 $\pm$ 13
Moist chamber storage	conus	96	(N = 99) 42.9	31.1	833 $\pm$ 19
	others	12	(N = 14) 51.4	40.9	1045 $\pm$ 12
Total	conus	60	(N = 55) 49.3	31.3	1022 $\pm$ 14
	others	42	(N = 41) 49.9	51.4	1111 $\pm$ 13

the method of Capella et al (1965) and Kaufman & Capella (1968). All corneas had been excised and placed in storage within six hours post mortem. The time limit for this method has been estimated at two years. In this group the frozen corneas had been in the eye bank from one day to three years. The total number of cryopreserved corneas was 42.

The average ages of the recipients and the donors in different preservation groups are seen in Tables I & II.

Table III  
Donor age and endothelial cell density

Age group of donors (years)	Total number of transplants	Mean age of donors	Mean age of recipients	Endothelial cell density (cells/mm <sup>2</sup> )
8-30	11	22.9	37.2	1424 ± 597
31-45	20	37.8	42.2	1401 ± 592
46-60	32	52.3	45.6	1105 ± 410
61-70	12	66.8	39.1	922 ± 253
>70	14	76.3	37.0	1011 ± 285
Donor not known	6		38.2	842 ± 359
Total	102	49.1 (96)	40.8	1169 ± 4.8

Donor age  
To evaluate the influence of donor age on graft endothelial cell density the patients were divided into four groups according to the age of the donor. In the first group the donors were ≤ 30 years whereas in the last two groups the donor ages were between 61 and 70 and over 70 years. In six cases donor age was unknown (Table I).

Photography of the corneal endothelium  
The endothelium of the clear transplant was photographed with a clinical specular microscope (Seyber Inc.). The image on the negative was × 100. This was checked by photographing a calibrated glass slide scored at 10 μm intervals. The prints had a final magnification of exactly × 500 (Fig. 2a, b, c). The endothelial cell density could be counted from the photographs by counting the number of cells in a known area which corresponded to 0.01 mm<sup>2</sup> in nature. In most cases three to five central graft cell areas were counted and the average was taken as the result.

Statistical analyses were made by Student's *t* test to ascertain the effects of various factors on the parameters studied.  
The patients were divided into different groups so that enough cases were found in each of them to make the statistical analyses possible. Also the different groups of cases were made so that the recommended or suggested critical times or ages could be checked and compared with the other possibilities for example critical cadaver time, postoperative period or donor age. In the text and tables the numeral values of cell densities ± standard deviations are to be given.

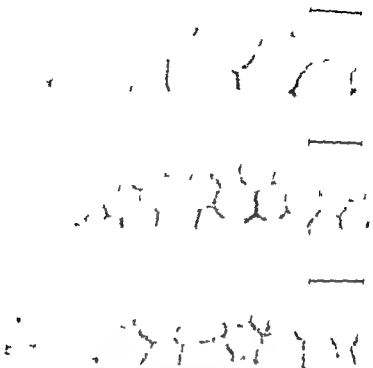


Fig 2

- A Central corneal endothelium on specular microscopv of a graft stored in *trans* solution for three hours. The mean cell density is 650 cells/mm<sup>2</sup> ( $\times 500$ ) Bar 50  $\mu$ .
- B Central corneal endothelium of the graft stored in M H medium for three days. The mean cell density is 2025 cells/mm<sup>2</sup> ( $\times 500$ ) Bar 50  $\mu$ .
- C Central corneal endothelium of a cryopreserved graft. The preservation time is 4 years. The mean cell density is 1425 cells/mm<sup>2</sup> ( $\times 500$ ) Bar 50  $\mu$ .

## Results

### Effect of corneal preservation on endothelial cell density (Tables I & II)

A total number of 102 clear penetrating transplants in 93 patients were photographed with the specular microscope. The average endothelial cell count of 102 transplants was  $1169 \pm 478$  cells/mm<sup>2</sup> with a mean donor age of 49 years.

The patients were divided into three different groups according to the method by which the donor corneas had been stored. In the control group with grafts

sh or after moist chamber storage the mean endothelial cell density was  $4 \pm 390$  cells/mm<sup>2</sup> the values ranging from 500 to 1800 cells/mm<sup>2</sup>. The number transplants was 41. The average recipient age was 40.1 and the average donor age 47.9 years.

In the grafts stored in M H medium the mean endothelial cell density was  $4 \pm 406$  cells/mm<sup>2</sup> the values ranging from 1000 to 2700 cells/mm<sup>2</sup>. In this group there were 19 patients. The average recipient age was 44.2 years and the average donor age 46.2 years.

Among the cryopreserved grafts the mean endothelial cell count was  $1102 \pm 308$  cells/mm<sup>2</sup> the values ranging from 400 to 2100 cells/mm<sup>2</sup>. This group contained 42 patients. The average recipient age was 40.0 years and the average donor age 41.4 years.

Corneal transplants stored in M H medium have more cells than cryopreserved grafts ( $P < 0.001$ ) or grafts stored in a moist chamber ( $P < 0.001$ ). The difference in endothelial cell density after cryopreservation and after moist chamber storage is also statistically significant ( $P < 0.001$ ).

The statistical significance of the differences in cell counts is even clearer when only the 60 keratoconus cases are considered (Table II).

#### Preservation time

In cryopreserved material the preservation times varied from one day to three years and two weeks. In material stored in M H medium from one to four days and in fresh corneas or material stored in a moist chamber from 0 to 48 h. Each group was divided into subgroups according to preservation time. No statistically significant difference could be detected between the different preservation times within groups of M H medium stored or cryopreserved grafts.

However the group with a moist chamber time of less than one hour (fresh corneas) was found to differ statistically from the others ( $P < 0.01$ ). No really maximal moist chamber time was observed to exist in the time interval from 1 to 48 h.

#### Effect of donor age

Table III shows the mean endothelial cell densities in the different donor age groups. The results were best in the two youngest groups with donors aged 40 years or less. In the first group with donors aged 8 to 30 years the endothelial cell count was  $1424 \pm 527$ /mm<sup>2</sup>. When the oldest donors were divided into two groups on both sides of 70 years there was no statistically significant difference between them.

An inverse correlation was found between the cell density of the graft and donor age the difference being statistically significant between donors younger and older than 40 years ( $P < 0.01$ ).

Table IV  
Effect of cadaver time (time from death to enucleation of eye) on graft endothelial cell density

Cadaver time (hours)	Number of transplants	Mean age of donors	Mean age of recipients	Endothelial cell density
0-1	33	(N = 31) 46.8	40.8	134 ± 33
1-4	22	(N = 20) 50.5	41.7	111 ± 14
4-6	22	47.5	39.9	109 ± 44
6-10	13	54.8	49.5	1030 ± 5.5
10-20	8	(N = 7) 12.1	51.6	40 ± 5
Unknown	4	(N = 3) 49.3	46.5	1115 ± 3.5

#### Effect of cadaver time (Table IV)

The endothelial cell density of the graft decreased with increase of cadaver time (time from death to enucleation). Such the best results were obtained when cadaver time was less than one hour. This group differed almost significantly ( $P < 0.05$ ) from the others, and when the cadaver time was more than 10 h the difference was statistically significant ( $P < 0.01$ ).

The mean cadaver times for grafts stored by different methods were all between 2½-6 h.

#### Effect of postoperative period (Table V)

The cell density showed a steady decrease with postoperative period. A statistically significant difference ( $P < 0.001$ ) in cell density was noticed if the postoperative

Table V  
Correlation between postoperative period and endothelial cell density of the graft

Postoperative period (years)	Number of transplants	Mean age of donors	Mean age of recipients (years)	Cell density (cells/mm <sup>2</sup> )
≤ ½	16	53.0	38.1	1515 ± 616
½-1	19	47.1	40.9	1441 ± 41
1-2	16	41.9	41.9	1311 ± 1.9
2-	25	(N = 23) 51.0	43.0	40 ± 5
1-11	26	(N = 23) 48.7	38.5	4.5 ± 1.2



od was more than two years as compared with the shorter postoperative times  
 rate of cell loss was much higher with graft endothelium than with normal  
 real endothelium during ageing  
 he follow up period for the grafts stored in M K medium averaged one year  
 opreserved grafts had a mean follow up period of two years and for moist  
 mber stored grafts it was 5½ years

## Discussion

mean endothelial cell density for all 102 transplants was  $1169 \pm 478$  cells/mm<sup>2</sup>  
 is corresponds well to the results of other authors for example Bourne &  
 ulman (1976b) found a mean cell density of 973 cells/mm<sup>2</sup> and Bron & Brown  
 (14) a mean cell density of 1125 cells/mm<sup>2</sup> Thus far the highest cell density  
 11 cells/mm<sup>2</sup> has been found in the series of Bourne et al (1977) of 14  
 an-cultured transplants In their study the follow up period was quite short and  
 can only guess at the long term values All studies concerning corneal endo-  
 bal cell density indicate that we lose quite a large number of endothelial cells  
 ring preservation and transplantation In my series with donors whose average  
 was 49½ years I found a mean endothelial cell loss of rather more than 50% as  
 mpared to the endothelial cell density of the normal cornea

The data of this investigation support the view that one of the best methods of  
 serving corneal endothelium for keratoplasty is intermediate term corneal  
 rage in McCarey Kaufman (M K) medium With preservation in M K medium  
 r transplants had many more endothelial cells ( $1847 \pm 456$  cells/mm<sup>2</sup>) than with  
 two other methods used in our hospital moist chamber storage ( $924 \pm 320$   
 ls/mm<sup>2</sup>) and cryopreservation ( $1102 \pm 308$  cells/mm<sup>2</sup>) The average follow up  
 od was three years which is supposed to be long enough for manifestation of  
 loss due to preservation and operation The average donor age in the different  
 ciation groups did not differ significantly So donor age cannot be the reason  
 for the variation in endothelial cell densities

Before the advent of cryopreservation and intermediate term preservation of  
 meas it was believed that material used fresh or after moist chamber storage  
 ould give the best corneal grafts However McCarey & Kaufman (1974) showed  
 electronmicroscopy and the temperature reversal viability test that storage of  
 aged corneas in M K medium gives better results than storage of fresh corneas  
 whole globes in a moist chamber Our study confirms that after moist chamber  
 orage transplants have significantly fewer endothelial cells than after storage in  
 M K medium

Our new finding was that the mean cell density of grafts was significantly higher after cryopreservation than after moist chamber storage. Presumably this is due to the difference in environment the corneal endothelium being exposed to. It is poisoned by degradation products of tissues when stored in a moist chamber, but when the cornea is trephined from the globe (Schimmelpfennig et al. 1971) there is very little fluid in the anterior chamber and metabolic degradation products do not accumulate there soon after the donor's death. According to our results, the time of corneal excision during the first hours postmortem is of the utmost importance.

The mean follow up period for grafts stored in moist chamber was less than for grafts which were cryopreserved. This fact may in some degree bias the difference in cell densities between these two groups, but does not explain the difference. The loss of endothelial cells after two years was very small (Table 1) and on the other hand the mean follow up period for cryopreserved grafts was also two years.

Even a relatively small number of endothelial cells can keep the corneal endothelium of normal thickness. This was observed in the transplants made after moist chamber storage and cryopreservation. With the M & H method we were able to trephine nearly twice as many endothelial cells as with the other two methods. In that respect intermediate term corneal storage using M & H medium seems superior to the two alternative methods.

A very interesting finding is that with cryopreserved material our transplants had more endothelial cells than those used after moist chamber storage, the difference being statistically significant. Thus the cryopreservation method appears to be the best possibility of corneal storage for long enough to allow selection of HLA compatible donor/recipient pairs. This increases the chances of successful corneal grafts in highly vascularized and so called 'high risk' patients (S. L. and al. 1976). Whether HLA compatibility affects endothelial cell density was not discussed in a further paper.

The cadaver time (time from death to enucleation or exsiccation) was found to influence the endothelial cell density of the grafts. Statistical analysis of the correlation between cadaver time and cell density showed that when the cornea was excised in less than one hour the cell density was significantly higher than in the other cadaver time groups. Further when cadaver time exceeded 10 h the cell density was significantly less than in the other groups. So far it has been known that 6 h is the critical cadaver time, but in this study I found that we can hope to obtain a good result up to 10 h post mortem. This is one reason for the better cell density in cryopreserved material compared with material stored in a moist chamber, that is the shorter cadaver time for cryopreserved material, which in our hospital has been 6 h.

An important factor for endothelial cell survival in corneal grafts seems to be

of the donor. The highest cell density was found in grafts from donors under 40 years of age (over 1400 cells/mm<sup>2</sup>). Bourne & Kaufman (1976b) likewise found an inverse correlation between donor age and the central endothelial cell density of the graft, and came to the conclusion that the younger the donor and the younger the recipient the more numerous will be the central endothelial cells.

One of the most important points that emerged in this analysis of the correlation between endothelial cell density and donor age was that after the age of 40 age made little or no difference. Among the older donor groups healthy material even from donors over 70 years-old gave results that were nearly as good as judged by endothelial cell density as corneas from donors in the age group 46-60 years. The density of  $1011 \pm 285$  for donors of over 70 years is as good as the mean cell density of around 1000 cells/mm<sup>2</sup> in many reports (Bron & Brown 1974, Bourne & Kaufman 1976b, Sato 1978).

The follow up period varied from 1 month to 11 years. It had a significant effect on the endothelial cell density of the graft. The cell density was highest in the transplants seen only six months after the operation. A statistically highly significant difference in the endothelial cell density of the graft was found two years postoperatively. It is known that endothelial cell densities decrease with age (Bourne & Kaufman 1976a, Laing et al. 1976a, b) but this analysis of the graft endothelium showed that postoperatively it loses cells at a much greater rate than normal endothelium. All this suggests that transplanted endothelium will be much more vulnerable to postoperative complications such as glaucoma, trauma and other eye diseases (Jones & Rice 1967). It also underlines the importance of transplanting as many endothelial cells as possible even though for some time after operation the cornea can be kept clear with a very small number of endothelial cells, about 13-15% of the normal cell density.

In this study the importance of three different preservation methods was evaluated. Long term cryopreservation was found to preserve more endothelial cells than moist chamber storage of eye globes, however M & K medium storage was superior to both the other methods. The long follow up also showed the importance of short cadaver time, donor age and the vulnerability of transplanted endothelial cells. The influence of histocompatibility on graft endothelial cell density remains to be studied.

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# THE SPECULAR MICROSCOPIC APPEARANCE OF CORNEAL GRAFT ENDOTHELIUM DURING AN ACUTE REJECTION EPISODE

## A Case Report

BY

THOMAS OLSEN

The specular microscopic appearance of a graft endothelium during a successfully treated acute endothelial rejection episode is reported. Changes with the increased thickness of the graft great disturbance in cellular morphology was observed. Individual cells were seen to appear swollen or with bright intracellular areas. Also abnormalities with respect to cellular junctions were seen with the occurrence of junctional complexes at which more than three cells met. The significance of these abnormalities is discussed. The morphological changes subsided gradually as the graft cleared and its thickness decreased. This study exemplifies the value of the non-contact specular microscopic technique which enables a thorough study of the cleared corneal endothelium without traumatizing the cornea and interfering with subsequent specular microscopic examinations.

**Key words:** specular microscopy—cornea—endothelium—rejection graft

The appearance of corneal endothelium in response to trauma has been observed by means of specular microscopy following various forms of insult such as air in the anterior chamber (Leibowitz et al 1974), osmotic dehydration of the endothelium (Sherrard 1976, 1978) and ultrasound (Olson et al 1978). Most of these studies have been carried out on animals. Little information is available on the human endothelium which may apply to the clinical situation.

The fact that *in vivo* specular microscopy of corneal endothelium requires a translucent cornea hinders to a certain extent a detailed study of the endothelium.

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um following trauma. The case presented here is an exception to that rule in that it was possible to study the specular microscopic appearance of a corneal graft epithelium shortly after onset of an acute rejection episode.

## Methods

Central corneal thickness was measured with a modified Haag Siret pachometer (Sperling & Siret 1971). Specular microscopy was performed using a non-contact technique described earlier (Olsen 1979).

### Case presentation

A 50-year-old man had penetrating keratoplasty done for the first time in 1974 due to a vascularized corneal scar after herpetic keratitis. The grafting was successful until one year later where the patient experienced an acute rejection. Two weeks passed before he attended for control. In spite of topical and systemic steroid treatment the graft remained oedematous and became vascularized. Retransplantation was undertaken January 1977. Postoperatively the graft was resutured in the lower left quadrant but otherwise the postoperative course was unremarkable. The case was included as No. 1 in the study of Ehlers & Bramsen (1978).

On November 7th 1978 the patient awoke in the morning with blurred vision. On examination the same day the graft was oedematous with epithelial bullae and scattered epithelial pigments on the posterior aspect of graft. No rejection line was present on this first day of onset but the cornea was thicker in the lower part than in the upper part. Systemic treatment with prednisone 30 mg daily, topical ultracortenol and subconjunctival decadron phosphate injections were started immediately. On this treatment graft thickness decreased

### CCT

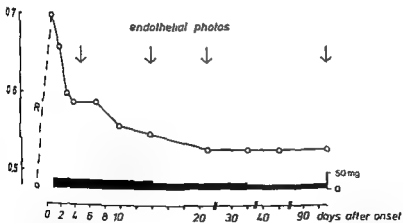


Fig. 1  
Diagram showing changes in central corneal thickness (CCT) (mm) of graft during an acute rejection episode. Black horizontal staircase line indicates systemic prednisone treatment.

gradually (Fig 1) On the 3rd day after onset of symptoms a typical irregular line across the lower part of graft This line was later broken up into smaller pieces the line the cornea was constantly thicker than above the line The cornea cleared in the ensuing weeks in which the graft gradually cleared Ten days after onset of symptoms precipitates were found with the slit lamp

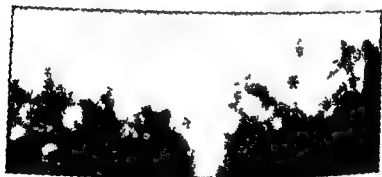


Fig 2

Endothelial cells of graft visible in the specular microscope 3 days after onset of symptoms. Top photo from upper part of graft Centre and bottom of graft is more heavily attacked part of graft The endothelial morphology is severely altered swelling of cells (examples shown by arrows) intracellular bright areas (arrows) abnormal cellular junctional complexes (circles) Horizontal bar = 10  $\mu$ m



## Specular microscopy

The first 4 days after onset of rejection episode it was not possible to observe and photograph the endothelium due to opacification of the graft.

1 day after onset (Fig 2)

The endothelial cells became discernible. At places no planar reflex from the endothelium was obtainable probably due to great distortion of endothelium. In the places in which the cells were visible some cells showed dark round intracellular areas which made the cells appear swollen as interpreted from the planar reflex from the center of the cells (arrows in Fig 2). Such cells also appeared more round than cells without this phenomenon. Other cells showed intracellular variation with respect to intensity of reflected light giving the impression of bright intracellular areas (asterisks in Fig 2). Cells were larger and more irregular in the lower part than in the upper part of cornea.

Abnormalities were also found with respect to cellular junctions. Normally cell boundaries meet each other at points where three cells meet. Cell meetings involving four or more cells are practically never seen in normal endothelia (Fig 6). At some places cells were found to meet four at a time (circles in Fig 2) thereby disturbing the regular hexagonal pattern.

14 days after onset (Fig 3)

The graft almost clear. The endothelial cells now reflected the light in a more even manner. Intracellular round dark areas were less pronounced but still intracellular bright areas were present. In Fig 3 abnormal cellular intersections are especially evident showing cell meetings involving more than three cells (circles in Fig 3).



Fig 3  
Endothelium of graft 14 days after onset of rejection. Photograph from lower part of graft. Circles indicate abnormal cellular junctions with dark spots occurring in the centre. Other symbols explained in Fig 2. Bar = 100  $\mu$ m.



Fig. 4

The specular microscopic appearance of graft 21 days after onset of rejection. Photomicrograph from lower part of graft. Size and shape of cells show considerable variation. Note cells with convex borders (arrows) appearing between larger cells. Bar = 1.0  $\mu$ m.



Fig. 5

93 days after onset of rejection the endothelium has reorganized into a more regular pattern. Top: upper part of graft. Bottom: lower part of graft. Note absence of previous cell difference in cell size from top to bottom of graft. Overall cell density is about the same which is clearly much lower than on previous photomicrographs. Bar = 1.0  $\mu$ m.

ly these abnormal junctional complexes consisted of joints of four cells but in 3 a junctional complex of five cells is also found (circle in right part of Fig 3) cell pattern is far from the normal hexagonal regular pattern the morphological changes being more pronounced in the lower than in the upper half of cornea.

1 day after onset (Fig 4)

The graft now appeared clear Cell size and shape showed still considerable variation with small cells with convex borders (arrows in Fig 4) appearing between the irregular cells Cell meetings where more than three cells met were only occasionally encountered (circle in Fig 4)

1 day after onset (Fig 5)

Graft thickness had now stabilized at a value somewhat above the thickness before onset of rejection. Size and shape of cells were now more uniform but not completely regular Whereas in the foregoing specular microscopic examinations



Fig 6

Specular microscopic appearance of normal corneal graft endothelium Top male 6 months endothelial density 1090 cells  $\text{mm}^2$  Bottom female 48 months endothelial density 1910 cells  $\text{mm}^2$  Bar indicates 100  $\mu\text{m}$

there was a considerable difference in size of cells from photoreceptor and lower part of graft this difference now was less apparent. The number of cells per unit area at this time was clearly much lower than that of endothelium (Fig. 6) and that of the first specular microscopic examination was difficult to give figures for this difference due to the large cell density observed within the graft. No clearcut abnormalities with respect to cellular intersections were found.

## Discussion

In interpreting the endothelial changes observed in this study it is prudent to regard them as partly due to the nature of the actual disease and partly due to secondary healing mechanisms of the endothelium. Graft rejection is generally believed to be a cell mediated immunological manifestation. With the use of the microscope it was not possible to observe any lymphocytes on the endothelium although precipitates were seen with the slit lamp. It was remarkable however that the cellular abnormalities in the endothelium were so widely dispersed and occurred at places without precipitates. Furthermore the rejection line seen at the slit lamp did not sharply divide the endothelium into an abnormal and a normal area. These non localized changes may be the result of soluble factors involved in the cellular hypersensitivity reaction for which a number of mediators are known to be involved (see David 1973) among them macrophage inhibitors and factors cytotoxic for target cells. Some of these factors may activate enzymes. Interestingly this process has been shown to result in swelling and rounding of target cells and their organelles followed by lysis of the cell (Leibowitz 1970). This may be the explanation for the apparent swelling of endothelial cells observed in the present study (Fig. 2).

The observed bright intracellular areas observed in the present study bear a certain resemblance to those events described in rabbits (Sherrard 1961, Leibowitz 1974) after air or osmotic dehydration of cornea. Sherrard described some of these events may progress to cell death after which a characteristic rosette formed by adjacent cells spreading to cover the defect (Sherrard 1961). Such rosettes formed after acute trauma have been described by others *in vivo* (David 1978) and *in vitro* (Sperling 1978). A characteristic feature for these rosettes is that the centre forms a point at which more than three cells meet depending on the number of cells surrounding the cell which has been destroyed. The complex of five cells described in Fig. 3 is in fact a rosette. The abnormal complexes of four cells which was of far more frequent occurrence are however not identical to such rosettes.

According to Sherrard (1976) and Olson (1978) these rosettes became reorganized within a few hours leaving a cell pattern almost indistinguishable from a normal endothelium. Based on *in vitro* studies Sperling (1978) noted that joint settings of four cells may stay longer than the rosettes and suggested that joints of four cells actually may be formed from the rosettes. The abnormal cellular junctions found in this study i.e. junctional complexes in which more than three cells meet may thus represent recent cell death.

It was not possible to make valid estimation of endothelial cell loss due to large variation in cell size from one part of the graft to another and the inability to photograph exactly the same area after periods of time. It may be suggested that a great cell loss occurred during the rejection episode (compare Fig. 3 with Fig. 5) and that the greatest cell loss occurred in the lower part of the graft as judged from the greater morphological changes and the greater thickness of graft in this region. Three months after onset (Fig. 5) however no marked difference in cell density is observed between upper and lower part of graft. This indicates that a certain drifting of cells towards regions of lower cell density has occurred a phenomenon which has also been described after cataract extraction (Rao et al. 1978).

The functional impairment of the endothelium accompanying the observed morphological changes was manifested through the changes in graft thickness: 1) The return of a clear thin graft 2-3 weeks after onset of rejection was accompanied by the specular microscope appearance of a planar endothelium with no major intracellular or intercellular abnormalities although a certain degree of cellular pleomorphism still existed (Fig. 4). This pleomorphism had reduced on the re-examination (compare Figs. 4 and 5). This indicates the process of active cell death and the immediate healing response is more deleterious for the barrier function of the endothelium than the process of reorganizing the cellular pattern from a state of pleomorphism to a more regular pattern.

In this study non-contact specular microscopy has proved very useful in studying morphologic alterations of endothelial cells following an insult. With the non-contact technique the whole endothelium can be scanned without traumatizing the endothelium and interfering with subsequent specular microscopic examinations.

### Acknowledgments

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# TOXICITY OF DIMETHYLSULFOXIDE (DMSO) TO HUMAN CORNEAL ENDOTHELIUM IN VITRO

BY

STEFFEN SPERLING and INGER S

Human cadaver corneas were obtained 10-19 h after death and incubated in organ culture for 20-28 h and exposed to 0-100% DMSO successively for 10 min at each concentration. Cell damage was recognized by morphological alterations during incubation and by cell detachment after expulsion of damaged cells. DMSO caused cell damage at 31°C. No cell damage was found at 4°C in minimum essential medium (Eagle) plus 10% human serum or when DMSO was dissolved in human albumin plus sucrose. DMSO was found to be toxic at 4°C in pure serum and in Dulbecco's medium.

**Key words:** cryopreservation - DMSO toxicity - endothelium - human cornea - latent injury - morphology - organ culture

Dimethylsulfoxide (DMSO) is the substance which so far has been most effective in protecting human corneal endothelium during deep freezing. O'Neill et al (1967) found by a technique of combined incubation and enzyme staining that canine and human endothelium was damaged in proportion to temperature, concentration of DMSO and time of exposure. Kaufman & Capella (1968) found by enzyme staining that 10% DMSO plus 10% sucrose in 20% human albumin was toxic to human corneal endothelium above 10°C and atoxic below this temperature. Schultz (1971) and Kanai et al (1972) found only slight ultrastructural changes in human endothelium after exposure to DMSO at 4°C in the media advocated by Kaufman & Capella (1968).

This study was undertaken after development of a sensitive method for detection of cell loss in human endothelium (Sperling 1978). The relation between DMSO toxicity, temperature of exposure and basic medium was evaluated.

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## Material and Methods

Human eyes were enucleated 10–12 h post mortem. Cadavers were stored 6–12 h at 4°C and at 10°C during the remaining post mortem time. Eyes with undetected intraocular air were rejected. In order to avoid bending of the corneas during enucleation, intraocular air was slowly injected in the vitreous until the ocular tension was found normal by palpitation. The eyes were rinsed in tap water. Corneas were excised with a 15 mm ring of sclera and stained by 0.3% trypan blue in 0.9% NaCl for one min. The corneas were then filled by 0.9% NaCl. The number of blue stained nuclei was counted in 10 random fields, each equivalent to 1.3 mm<sup>2</sup> on cornea after random setting of the microscope. The corneas were flattened 6 mm of the cornea. The intercellular borders and the endothelial borders were obtained and three were selected for each cornea. For each photograph the cells in one test area, equivalent to 0.03 mm<sup>2</sup> on cornea were counted. The methods of counting and selection of photographs and test areas described by Sperling and Gundersen (1978) were applied.

In 64 corneas from patients aged 13–91 years less than one per cent blue stained endothelial cells were found. Patient age and time between death and inclusion in the study are shown in Fig. 1. These corneas were organ cultured for 20–98 h before use in the experiments. The technique described by Sperling (1979) was applied. Organ culture at 31°C in a minimum essential medium with Earle's salts, L-glutamine, NaHCO<sub>3</sub>, eight per cent fetal calf serum and ten per cent fetal calf serum. In the following this medium is denoted MEM.

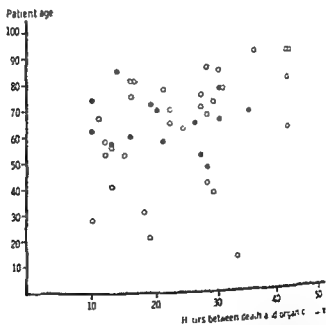


Fig. 1

Patient age and time between death and organ culture indicated for each of the 64 corneas included in this study. An open circle represents one cornea. A closed circle represents two corneas from the same patient.



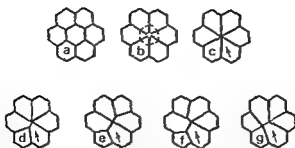


Fig 2

a-c Formation of a joint meeting of six originally hexagonal cells after loss of a central seventh cell

d-g Transformation to joint meetings of four cells  
Arrows indicate reformation figure centres

all experiments with DMSO corneas were subjected to 0, 4, 6, 8, 11, 4 and 9% DMSO routinely for 10 min in each concentration. The toxicity of DMSO from Merck (für mikroskopie, Lvasol 2900) and from Pierce (Silylation grade) was evaluated. DMSO at 4°C was evaluated in MEM, in fetal calf serum, in Dulbecco's phosphate buffered medium with 10% serum (Dulbecco & Vogt 1954) and in salt poor human albumin (10% from Rhodia 25°C Cutter). Recent cell loss was recognized as described by Sperling (1979). Staining of the endothelial borders in alizarin red and counting of reformation figures (cell constellation occurring during reformation of the endothelial pattern after cell loss). Reformation figures are shown in Fig 2b-g. From each cornea four photographs were obtained and three were used for counts. On each photograph reformation figure centres (Fig 2) were counted in 16 frames each equalling 0.076 mm<sup>2</sup> on cornea and the cells were counted in one frame equalling 0.03 mm<sup>2</sup>. Damage after exposure to DMSO was expressed as reformation figure centres (Fig 2) in per cent of cells per area.

## Results

between exposure and damage

ten corneas were exposed to DMSO (Pierce) in MEM at 31°C. Four of these corneas were evaluated immediately after the exposure and 16 were evaluated at 1, 2, 4 and 24 h of organ culture at 31°C. Four control corneas were organ cultured for 24 h without exposure to DMSO.

The percentages of reformation figures are indicated in Fig 3. The largest numbers of reformation figures occurred one and two hours after exposure to DMSO. Very few reformation figures were found immediately after and four hours after the exposure. After 24 h of organ culture more reformation figures occurred in the corneas subjected to DMSO than in the controls.

mucosal epithelium in tissue culture (Jepsen 1978) DMSO (Menzel & Sperling 1974) were originally used as basic cryoprotective media by Mueller & Smith (1964) by Kaufman & Capella (1968) Dulbecco's medium was chosen because of the survival of bovine embryos stored and frozen in this medium (Truman & Sperling 1976)

In the present study DMSO at 31°C caused an increased endothelial death at 2 and 24 h after the exposure while this was not the case immediately after exposure Latent damage was also found in human and canine corneas by O'Neill et al (1967) and by Sperling (1976) in bovine endothelium The time between exposure to DMSO and the occurrence of cell death suggests that it interferes with the cellular metabolism The results of this study show two different sites of interference One leading to irreversible cell injury immediately after the exposure and the other leading to a lowered cell survival in organ culture more than 4 h after the exposure to DMSO

In this study DMSO was found to be toxic at 31°C and alone or dissolved in MEM Increasing toxicity of DMSO with temperature was found in canine and human endothelium by O'Neill et al (1967) In an earlier study on bovine corneas (Sperling 1976) increasing toxicity of DMSO with temperature was not found

In the present study very slight cell damage was found when corneas were exposed to DMSO at 4°C in MEM or in albumin plus sucrose solution The toxicity to human endothelium at 4°C in albumin plus sucrose was found by O'Neill (1971) and by Kanai et al (1972) Parallel observations on rabbit endothelium have been reported by Kaufman & Capella (1968) van Horn et al (1971) Sperling et al (1973) Geroski & Edelhauser (1974) and by Basta et al (1975) The toxicity to human endothelium at 2°C in a medium comparable to MEM was found by Mueller (1968)

In the present study some cell damage was found in pure serum at 4°C and in Dulbecco's medium Damage occurred when DMSO was added to either of these media Varied DMSO toxicity in different basic media was also found in bovine corneas (Sperling 1974-1976) An explanation for this phenomenon could be that the toxicity of DMSO only becomes manifest in a suboptimal basic culture medium would parallel earlier observations of additional effects of unrelated mechanical trauma (Sperling 1974-1976)

## Conclusions

The toxicity of DMSO increases with temperature For several quarters of time the toxicity is very low at 4°C when DMSO is dissolved in basic media

in or Dextran DMSO exerts latent damage to corneal endothelium This suggests that DMSO toxicity should not be evaluated immediately after the exposure to DMSO

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## REFSUM'S DISEASE

### Eye manifestations in a patient treated with low phytol low phytanic acid diet

BY

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The cardinal eye symptoms of Refsum's disease are night blindness, retinal pigmentary degeneration and constriction of the visual fields. Similarities with or differences from retinitis pigmentosa are discussed. A 39-year-old male has had manifestations of Refsum's disease from the age of 7 years and has been on a low phytol low phytanic acid diet for the last 13 years. Peripheral ring scotomas were present. Some reduction of the visual fields has been recorded but only in the far periphery. The central field has not been significantly constricted during a 7 year period. Very good visual functions were found within this area. All cone mechanisms were functioning at a normal level. A moderately reduced sensitivity level of the rod mechanism could be explained in a great measure by poor pupillary dilation in the dark. In this patient minimal or no progression of the visual findings was apparent. Probably an effect of treatment, there is little resemblance with ordinary retinitis pigmentosa. ERG showed moderate abnormality. Normal conducting time was found by VEP. With fluorescein angiography a central area of normal appearance was sharply outlined in contrast to marked degenerations in paracentral regions.

*Keywords:* retinal pigmentary degeneration - visual fields - colour vision - cone mechanisms - ERG - VEP - fluorescein angiography - dietary treatment

In reports of Refsum's disease eye manifestations are generally described as a secondary retinal degeneration of the retinitis pigmentosa type with night blindness as a characteristic and early symptom. A closer evaluation of the photoreceptors by selective registration of their response functions may be of particular interest in order to see in which way Refsum's disease accords with or differs from the retinitis pigmentosa type of retinal dystrophy.

Accumulation of phytanic acid in the serum is a characteristic of Refsum's disease and is probably the cause of some important neurological disorders. Phytanic acid is not an endogenous product but is supplied with the food mainly as phytol and as its precursor phytol. As a consequence treatment with a low phytol diet and a low acid diet has been started (Eldjarn et al. 1966) whereby regression of several symptoms has been recorded though not visual and hearing loss (Eldjarn & Refsum 1977). We report here the findings in a patient with Refsum's disease over a 13 year long period of dietary treatment.

### The patient

**H. M.** a male 39 years of age was seen at the hospital for the first time at the age of 7 years. Six months previously slight paresis had developed in the lower limbs. Subsequently ataxia, ichthyosis and a slight hearing defect were also recorded. At the first ophthalmological examination a convergent squint of the left eye and moderate astigmatism and amblyopia was found. Normal pupillary reactions were noticed. There was nothing to remark from ophthalmoscopy. One year later a ring and pepper pigmentation was noticed in the central part of the fundus. The diagnosis "heredopathia atactica polyneuriformis" was made by Professor Schanz when the patient was 8 years of age.

**Course.** The pigmentation of the fundus became more distinct at the age of 10 years. At the age of 27 years numerous pigment spots of the retinitis pigmentosa type were found in a midperipheral zone of the fundus. By perimetry a ring scotoma was recorded on the right side and an incomplete ring scotoma on the left side. A special diet low in phytanic acid was instituted from 1965 at the age of 22 years. A clear therapeutic effect was observed. Reduced night vision was noticed at the age of 8 years. The patient admits that his night vision is reduced only to a moderate degree as he has never had problems finding his way at night. His experience with colours has always been good. The visual acuity of both eyes has been normal (6/6).

## Methods

rk adaptation curves have been recorded with the Goldmann Wecker adaptometer. Metric examinations under varying adapting conditions were performed with a modified Goldmann perimeter (Hansen 1974; Hansen & Seim 1978). Near monochromatic interference filters (half band width 10–15 nm) were used. The angular size of the target is expressed in degrees and minutes. As the test target is slightly elliptic the diameter of the equivalent circular target comprising the same area is indicated. Background illumination is measured with a luxmeter (Hartmann & Brown EBL\3) in the bottom of the bowl. The luminance of the test target is calculated as reflected energy at the level of the sphere.

Colour vision was examined with pseudo-isochromatic chart series (the Ishihara charts 14th edition) and the AO-HRR charts. Besides the Farnsworth tritan chart, the Farnsworth D 15 test and the Farnsworth 100-Hue test were used. Induced colour contrasts were recorded with a series of tissue paper contrast charts (Hansen 1976). Anomaloscope examinations were performed with a Nagel anomaloscope type I.

Electro-retinographic examination was done by using a Lovac ERG contact lens (Medical Workshop) with a refractive power of +100 D having a corneal electrode and a conjunctival reference electrode. By means of a signal averager the single flash-evoked ERG response was recorded as the average of 50 single flash-evoked responses. The photosimulator was a Grass 4 strobe scope with 10  $\mu$ s long electronic flashes. The intensity of the flashes was set at 8 for photopic recording and at 1 for the scotopic.

Visual evoked response (VER). Flash train-evoked response patterns were recorded from the scalp with midline derivation O<sub>1</sub>-C<sub>z</sub>. Identical trains consisting of 40 flashes at 10 Hz were presented monocularly and the average response to 50 trains was recorded. By omitting one flash in the steady state part of the response pattern it was possible to measure the latency as the time elapsed from the omitted flash to the cessation of the periodicity.

Dynamic tonometry. Pulse synchronous variations in intraocular pressure were registered by a method described by Hørvén (1970).

A Nikon wide angle (45°) fundus camera was used for the fundus photography and the fluorescein angiography.

## Ophthalmological findings

The patient had central and stable fixation with his right eye. There was unstable fixation with the left eye but no obvious strabismus. The objective findings in the two eyes were identical. There was no nystagmus. Strands of persistent pupillary membranes were present. The pupils were small measuring 3 mm in diameter in primary illumination (100 lux). Direct and consensual reaction to light was normal. There was slight reaction to accommodation. The pupils dilated poorly in darkness. At 0 minutes in the dark the pupillary diameter was 4.5 mm (registered photographically). After repeated application of cyclogyl and metaoedrine the pupillary diameter measured 6 mm. Except for very delicate granular opacities in the posterior cortex and stroma the lenses were clear and so was the vitreous body. The optic discs were well marked, their colour within the normal variance.

Normally calibrated retinal vessels were seen in the central fundus. The peripapillary vessels were moderately attenuated. There was a peripapillary atrophic halo. Extended and marked degenerations were seen in the fundus near the equator including the upper part of the macula. The fovea and lower part of the macula had a normal appearance (Fig. 1 A). The degenerative changes indicated atrophy of the retina, depigmentation of the pigment epithelium and loss of choroidal vessels.



Fig. 1

- A Fundusphoto of the left eye showing peripapillary atrophic halo and extensive degenerative changes also affecting the upper part of the macula.  
 B Fluorescein angiogram in the late venous phase left eye. Hypofluorescence at the fovea. Marked degenerative changes as reflected by increased leakage and fluorescence in the fovea as normal.





vessels. The greater choroidal vessels had a normal appearance. Grey pigmentary areas and spots of pigments of bone corpuscular and ovular shape were seen towards the equator region. More peripherally the fundus appearance changed from normal to atrophic areas.

Fluorescein angiography (Fig. 1 B) verified the finding of normally calibrated retinal vessels. The retinal circulatory phases were normal and so was the choroidal flush. There was hypofluorescence of the optic disc suggesting some optic atrophy which was not revealed by the ophthalmoscopic examination. The degenerative changes were clearly outlined, especially those of the pigment epithelium, thus giving a sharp border between the degenerated and the normal fundus.

Visual acuity was 6/6 on the right eye and finger counting 1/2 m on the left eye.

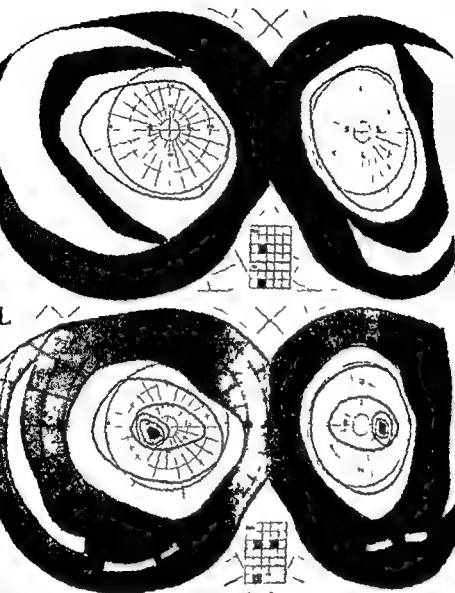


Fig. 2

Registrations of the visual fields with a 7 years interval.  
Recorded in 1971 (upper) and 1978 (lower)

Visual fields recorded in the Goldmann perimeter (Fig. 2) show broad peripheral ring scotoma reaching the periphery on the nasal side. Compared with the recording made in 1971, 7 years previously, there is a greater loss in the periphery leaving a smaller crescent of the field. However, the visual fields were not significantly changed in the central part.

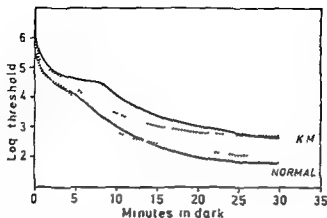


Fig 3

The dark adaptation curve of K M. The normal mean values of the apparatus + 2 s.d. are shown. The dotted lines indicate the performance of a normal person with ordinary pupils (lower curve) and 2 h after instillation of pilocarpine (2%) in the eyes (upper curve) the pupillary diameter being 2½ mm.

### Special examinations

#### Colour vision

There was a normal performance with the Ishihara's test, the AO-HRR test, the Farnsworth's D-15 test and the Farnsworth's titman chart. With the Farnsworth Munsell's 100-Hue test only a few chips were confused (making 26 error scores). All the figures in a series of tissue paper contrast test charts could be seen. With the Nagel anomaloscope he had precise settings within the normal range (41.5-43/16).

#### Dark adaptation

The course of dark adaptation has been repeatedly examined during the last 8 years showing essentially the same results. There is a rather good response in the first phase and a moderate elevation of the thresholds during the second phase, the final thresholds being about 1 log unit above the mean normal value (Fig 9). However, the poor dilation of the patient's pupils in darkness (maximally 4½ mm) influences the result. The effect of miosis on the dark adaptation curve is shown by a normal observer. Two curves were registered, one with normal pupils and one with artificial miosis (2½ mm) caused by installation of pilocarpine (2%) in the eyes. In the latter case elevated light thresholds were recorded, the final thresholds being at the same level as those obtained for the patient.

#### Quantitative perimetry

Static perimetry registered on the white standard background shows a high threshold sensitivity within the central area, about 20° of eccentricity, though with

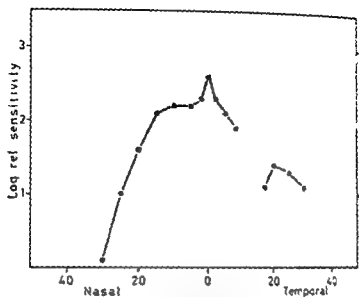


Fig. 4

Static perimetry on standard white background ( $10 \text{ cd m}^{-2}$ ) with eye (X-axis) 11  
Shaded area indicates the normal variation (mean  $\pm 1\sigma$ )

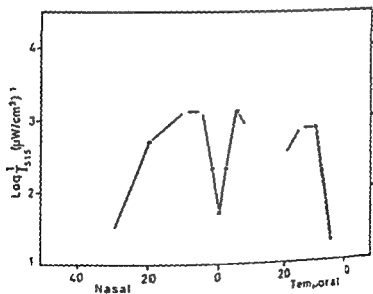


Fig. 5

Static perimetry during total dark adaptation recorded with a given eye  
( $\lambda_{\text{max}} = 510 \text{ nm}$ ) subtending  $27^\circ$  angular diameter. Shaded area indicates the  
normal variation (mean  $\pm 1\sigma$ ) for 5 normal subjects.

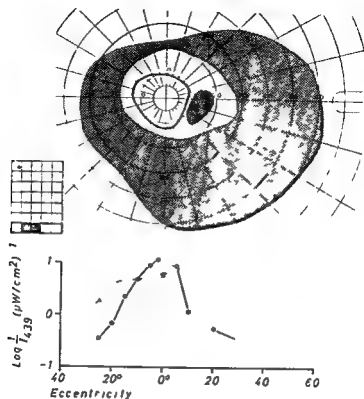


Fig 6

kinetic and static perimetry against a yellow background (low pressure Na lamp  $\lambda = 589 \text{ nm}$ ,  $100 \text{ lux}$ ). A blue target ( $\lambda_{\text{max}} = 439 \text{ nm}$ ) subtending  $1.47^\circ$  angular diameter is used. The greatest isopter corresponds to the threshold sensitivity  $= 0.69 (\mu\text{W}/\text{cm}^2)^{-1}$ . Dark area indicates the field loss in relation to a normal person of the same age of whom the static perimetry curve is also indicated (supplied line).

reduced sensitivity near the blind spot (Fig. 4). During total dark adaptation the static perimetry curve is reduced by about 1 log unit in the central part of the field and by more than 2 log units outside the  $20^\circ$  nasal and  $30^\circ$  temporal position (Fig. 5). Spectral sensitivity measurements against a yellow background show a good corresponding blue mechanism with a peak sensitivity at about  $439 \text{ nm}$ . A static perimetry curve obtained with the blue target ( $\lambda_{\text{max}} = 439 \text{ nm}$ ) demonstrates a high threshold sensitivity of the blue cone mechanism in the central region within the  $10^\circ$  nasal and  $10^\circ$  temporal position. The extent of the visual field of the blue mechanism is more clearly shown by the kinetic perimetry record obtained with the same target against a yellow background (Fig. 6).

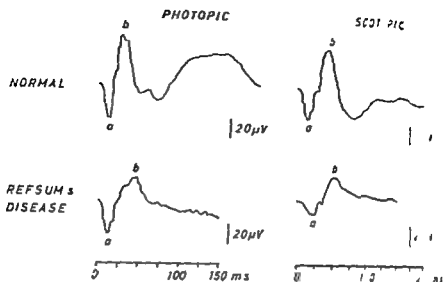


Fig 7

ERG records compared with those of a normal person under the same conditions

### Electroretinography

The photopic ERG showed subnormal amplitudes: the a wave measured 12 µV and the b wave 22 µV (Fig 7). The peak time of the a wave 125 ms is normal while the peak time of the b-wave 475 ms is significantly prolonged (mean normal value = 37 ms). Oscillatory potentials are present in the ascending phase of the b-wave but with reduced amplitudes. The scotopic ERG is also subnormal. The amplitude of the a wave is 12 µV and that of the b-wave 20 µV. The latency (take-off) of the a wave is 75 ms (prolonged) and prolonged peak time is 125 ms, the a- and b-wave being 20 and 10 ms respectively.

### Visual evoked response

The average brain-evoked response patterns from the right and left eye respectively showed well preserved periodicity in the steady state part. The latency recorded was 35 ms on each eye which is within the normal limits.

### Dynamic tonometry

The corneal indentation pulse amplitudes were moderately reduced, mean 4.5 µm on each eye (mean normal value found by Hansen (1970) is 9.5 µm).

### *Other findings*

Electromyography (EMG) and nerve conduction velocity

EMG has been repeatedly recorded during the last 8 years of the observation period. In 1970 and 1971 the EMG findings were markedly abnormal with very abundant denervation activity in all the muscle groups examined in the upper as well as the lower extremities. The occurrence of denervation activity has a decreasing tendency. Thus on the last examination in 1978 no denervation activity was found.

In the first period after the start of the dietary treatment (in 1962) the conduction velocity of the peripheral nerves more than doubled. It increased from 7 to 19 m/s in the ulnar nerve as reported by Refsum & Munthe-Kaas (1966) and Stokke (1969) the normal value being about 50 m/s. On later examinations 1968–1978 the values varied between 25 and 30 m/s.

Neurological findings

A peripheral neuropathy was present showing atrophy of the muscles of the extremities and incomplete loss of superficial and deep sensory qualities. Besides there was anosmia and moderate neurogenic hearing loss of the cochlear type. On comparison with previous neurological examinations it is obvious that the patient's condition has improved. The improvement was most clearly seen in muscular function during the first two years after the start of dietary treatment concomitant with a fall in serum phytanic acid to a normal level. While the muscle reflexes in the upper extremities prior to the dietary treatment were abolished they can now be elicited.

### Discussion

The chief manifestations of hereditary ataxia polyneuriformis are the eye symptoms and the neurological disorders comprising chronic polyneuropathy and ataxia. In most cases cardiac disturbances and hearing loss also occur. The disease is rare. In a review by Refsum (1977) the total reported cases were 73. Unlike patients with retinitis pigmentosa patients do not generally live until old age.

Since the introduction of the low phytanic acid diet, the prognostic situation has been considerably changed. Being one of the first patients treated by Eldjarn et al (1966) our patient has now been on a low phytanic acid diet for 13 years. The manifestations of the disease may therefore be much modified.

The cardinal eye symptoms of Refsum's disease are night blindness, constricted visual fields and a pigmentary retinopathy resembling retinitis pigmentosa. A great significance has been ascribed to the night blindness. However, in reports of Refsum's disease night blindness has been demonstrated in a few cases by adiptometric registration. Edström et al (1959) found photometric examination using the Cullstrand's method minimum luminance  $10 \times 10^{-3}$  lux in one patient, a definitely hemeralopic value. Sel et al (1971) described adiptometric curves as "indiscutably elevated", "certainly elevated" or "slightly elevated". Night blindness is said also to be present in our patient at the beginning of the disease. In our patient night blindness had developed at the age of 9 years when the diagnosis was made. However, the night blindness described in this patient was of moderate degree and was not pronounced at the age of 26 years when the dietary treatment was started. The dark adaptation as recorded during the following years showed a moderate elevation of the thresholds. By evaluating the dark adaptation curves of this patient the dilation of the pupils in darkness should be taken into account. His dark thresholds are close to those found in a normal person with artificial pupils. Therefore, at the present stage our patient does not demonstrate any marked defect of night vision. As miosis and poor dilation of the pupils are common findings in Refsum's disease (Refsum 1977) this should be allowed for in the evaluation of the dark adaptation.

Pathological pigmentation in the fundus had developed during the first 10 years of observation. Rapid development of pigment in Refsum's disease has been described by Edström et al (1959) who initially found a bilateral retinal detachment, protrusion opacities in the vitreous bodies and precipitates in the anterior chamber of one eye. This was followed by retinal pigmentation one month later when the oedema had been absorbed. This suggests a chorioretinitic type of reaction as the initial stage of Refsum's disease which may possibly be induced by physical factors causing a toxic effect. Discrete homogenous flake in the anterior chamber and lipid corpuscles in the vitreous was described in a child by Nordhagen & C (1964) without however any other signs of uveitis.

Pigmentation in Refsum's disease most commonly appears as a fine granular pigmentation ("salt and pepper") while the bone corpuscle type characteristic for retinitis pigmentosa is more infrequent. As in retinitis pigmentosa retinal vessels and discoloured optic discs have been described as typical findings in advanced cases of Refsum's disease. This was not found in our patient except for some slight attenuation of the retinal vessels in the periphery.

Fluorescein angiography demonstrates defects of the pigment epithelium and atrophy around the optic disc and peripheral degeneration. Gellera & B (1969) in 2 patients found a normal passage of dye through the choroid.



circulation a finding which is in accordance with the histopathological reports Alfandari (1971) in 4 patients found no features characteristic of Refsum's disease other than those seen in tapeto-retinal degenerations on examination with fluorescein angiography Dry et al (1976) in two new cases found diffuse changes in the pigment epithelium with peripheral pigmentary migration and important atrophic zones in the chorioretina

A moderate reduction of the corneal indentation pulse amplitudes was registered in our patient indicating reduced choroidal circulation. Low pulse amplitudes are a typical finding in retinitis pigmentosa (Hörven 1970). In this case it may be consistent with an affected choroidal circulation in the periphery while a normal circulation may still be present in the central area. Some optic atrophy could be traced by fluorescein angiography. The normal latency found by VER however is indicative of a normal myelination of the optic nerve or only a slight degree of demyelination.

As phytanic acid by *in vitro* experiments in peripheral nerves produces demyelination (Dubois Daleq et al 1972) and demyelination actually has been found on autopsy (Goulon et al 1977) a prolonged latency time is to be expected in patients with Refsum's disease. Optic atrophy with blindness has also been reported. However no reports of VER examinations have been given from untreated nor from treated cases of Refsum's disease.

There have been no reports of colour vision in patients with Refsum's disease except for one case reported by Alfandari (1971) having a very slight blue yellow defect as found by the 100 Hue test. In retinitis pigmentosa blue yellow discrimination is most often affected (Vernesi 1974). The finding of normal colour vision in our patient indicates a good cone function at a normal sensitivity level. This was confirmed by the static perimetry in white light by registering chiefly the long wavelength mechanisms. A separate registration of blue cone function likewise demonstrates a normal level of sensitivity. The typical finding in retinitis pigmentosa is a lack of rod response and a general depression of threshold sensitivity for all cone mechanisms but especially for the blue mechanism this being parallel to a blue type of colour vision defect (Hansen 1977). Such changes may even be seen in early stages of retinitis pigmentosa.

In most cases of Refsum's disease the ERG is extinguished. Schott et al (1968) and Rougier (1970) describing the same patients found extinguished ERG in one patient and greatly reduced ERG (to 50%) in an affected brother 14 years of age. A younger slightly affected brother had normal ERG. In our case a subnormal ERG was found. The increase in latency time of the a- and b-waves is consistent with those found in dominantly transmitted pigmentary retinopathies (Babel et al 1977) where the oscillatory potentials are also preserved as in our case.

The findings in our patient are only in part consistent with retinitis pigmentosa.

Seen together with the complete loss of receptor functions in Kretzschmar et al. (1976) there are remarkably good functioning receptors in the preserved retina feature not typical for pigmentary dystrophy. Most probably the preserved function in this patient is a consequence of the treatment with a low protein diet. Both in retinitis pigmentosa and in Refsum's disease without treatment there will be a progression of the visual disorder and a constriction of the visual field, especially in the expected. In our patient the progression has apparently been arrested. A definite improvement in other respects has been observed as is shown by the improved conducting rate, the EMG response as well as by the clinical improvement of the ataxia and ataxia. Only a few patients with Refsum's disease have been observed to respond to treatment. 2 patients of Eldjarn et al. (1976) including our patient, 2 patients of Lundberg et al. (1972) and 2 patients of Dry et al. (1974) examined over 5 years. Despite of regression of neurological symptoms it is generally supposed that the visual failure and the hearing loss are not modified by the treatment. In our patient there has been no regression of the visual field defects and his hearing defect remains unaltered. However, approximately normal visual functions are present in the central field. No further constriction has occurred as could otherwise be expected. This certainly is a great therapeutic gain.

### Acknowledgment

We are indebted to Professor S. Refsum for critical reading of the manuscript and for valuable suggestions.

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# CHARACTERISTICS OF OPTIC DISC IN HEALTHY SCHOOL CHILDREN

BY

H. ERKKILÄ and L. LAATIKAINEN

The ophthalmoscopic features of the optic discs were studied in a series of 41 non selected school children representing four age groups from 10 to 13 years. The distribution of the cup disc diameter ratios (C/D) showed that in the majority of the eyes (58.8%) the ratio was 0.0-0.3 independently of the age group. The highest ratio recorded 0.7 was found in two eyes of the series. An asymmetry of 0.2 or more in the C/D ratios of the eyes was found in 5.4% of the children studied. The correlation between C/D ratios and the refractive error was not statistically significant although C/D ratios of 0.4 or more were significantly commoner in myopia of  $-2.0$  D or more than in the other eyes. A preponderance of large C/D ratios was also found in children with a birth weight of 2500 g or less but the difference from the distribution in the other series was not significant.

Cilioretinal arteries were detected in 17.7% of the eyes and in 37% of the subjects studied. In 8.1% of the children the condition was bilateral. In children with a birth weights of 2500 g or less cilioretinal arteries were found in more than half of the cases.

*Key words:* optic disc - optic disc cup - cup disc ratio - cilioretinal arteries - birth weight - refraction - epidemiology - children - school health.

The normal optic disc shows great physiologic variation between one child and another. The role of inheritance in determining the cup disc diameter ratio (C/D ratio) was established by Armarh (1967). Later it was shown that the cup disc ratio varies with the size of the eyeball and the size of the optic disc (Tennant & Fawcett 1977).

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99 Bengtsson 1976 Fledelius 1978) This lead to doubts regarding the value of the wide spread use of C/D ratio in the follow up patients with ocular hypertension

In newborn infants large cups and asymmetry in C/D ratio are uncommon (Khodadoust et al 1968 Richardson 1968) In late childhood considerable changes occur in refraction (Sorsby et al 1957 Laatikainen & Erkkila 1979) and it has been estimated that after the age of 11 years the eye can still grow 0.2–0.3 mm a year up to and even after the age of puberty (Sorsby et al 1957) Thus changes in the optic discs might also be expected in late childhood Therefore in connection with a general ophthalmological survey of healthy school children presented in a separate paper (Laatikainen & Erkkila 1979) it was decided to study the C/D ratio and other features of the optic disc in children

## Subjects and Methods

A non selected group of 411 comprehensive school children comprising 23 whole classes and representing four age groups from 7 to 15 years were examined The distribution of the pupils is shown in Table I

The children underwent a thorough clinical ophthalmological examination Refraction was determined after cycloplegia (40–60 min after instillation of 1% cyclopentolate twice in each eye) C/D ratio was estimated using direct ophthalmoscopy by comparing the horizontal diameter of the cup to that of the optic disc The existence of optic disc drusen myopic conus and vascular or other abnormalities as well as the number and size of chorioretinal arteries were recorded

## Results

C/D ratio  
C/D ratio was analyzed in 814 eyes (in eight eyes this data had not been recorded) The cup was absent or minimal (C/D = 0.0–0.1) in 191 eyes (23.5%) In the majority 479 eyes (58.8%) the ratio was 0.2–0.3 and in 144 eyes (17.7%) C/D was

*Table I*  
*Age and number of subjects examined*

Age (years)	Number
7 – 8	81
9 – 10	109
11 – 12	111
14 – 15	110
Total number	411

Table II  
C/D ratio in the various age groups.

C/D ratio	Age group								Total	
	7-8		9-10		11-14		14-15			
	No	%	No	%	No	%	No	%	No	%
0.0-0.1	27	16.7	50	22.9	61	24.5	33	41	171	25
0.2-0.3	112	69.1	124	56.9	117	54.7	106	51.3	459	44
0.4-0.6	23	14.2	44	20.2	34	15.9	41	18.6	142	14
≥ 0.7	-	-	-	-	2	0.9	-	-	2	0.1
Total	162	100.0	218	100.0	214	100.0	180	100.0	556	100.0

0.4 or more in two of them 0.7. The C/D ratios showed no statistical differences between the various age groups (Table II).

In most cases C/D ratio was symmetrical in both eyes. In 19 of 401 cases (4.7%) the difference between the eyes was 0.2 and in 2 cases (0.5%) more than 0.2.

Large cups (C/D ≥ 0.4) were proportionally more common in myopic than in other eyes (Table III) but the correlation between C/D ratio and refraction was not statistically significant ( $\chi^2 = 5.307$ ,  $0.2 < P < 0.3$ ). In eyes with myopia of  $\geq 0.5$  D or more C/D ratio of 0.4 was found in 9 of 24 eyes (37.5%) which differs significantly from the other eyes ( $\chi^2 = 6.664$ ,  $0.005 < P < 0.01$ ). The correlation of

Table III  
Correlation of C/D ratio and refraction of the eyes

C/D ratio	Refraction (dioptries)					
	≥ +2.0		+1.25 - -0.25		≤ -0.5 (≥ -1.0)	
	No	%	No	%	No	%
0.0-0.1	20	22.7	152	23.5	19 (5)	5.4
0.2-0.3	51	57.6	388	60.1	40 (10)	30.0
≥ 0.4	17	19.3	106	16.4	91 (4)	24.6
Total	88	100.0	646	100.0	50 (4)	100.0

Table IV

Presence and size (large medium small) of cilioretinal arteries

Cilioretinal artery	Eyes with cilioretinal arteries	
	No	%
Large	21	9.8
Medium	35	4.7
Small	75	10.1
Total	131	17.7

Large cupping and myopic conus was highly significant of the 19 eyes where conus was established C/D ratio of 0.4 or more was found in 11 ( $\chi^2 = 21.096$   $P < 0.0005$ )

Findings in eighteen children with a birth weight of 2500 g or less were analyzed separately. In 10 of the 36 eyes (27.8%) C/D was 0.4 or more, two of these were myopic ( $\approx -0.5$  D) and four were hyperopic ( $\approx +2.0$  D). The prevalence of large cups in children with low birth weight did not differ significantly from that in the whole group ( $\chi^2 = 2.631$   $0.1 < P < 0.2$ )

#### Disc vessels and cilioretinal arteries

An aberrant vascular pattern such as early branching of the retinal vessels on the optic disc was seen in six eyes.

Cilioretinal arteries were detected in 131 of 740 eyes studied (17.7%) (Table IV). In the 74 eyes of the 37 first examined children this data had not been recorded. In 30 of the 370 children (8.1%) the cilioretinal arteries were bilateral and in 71 children (19.2%) unilateral. Thus 101 of 370 children (27.3%) had cilioretinal arteries in one or both eyes.

In 90 eyes (2.7%) one or two large cilioretinal arteries were found on the temporal side of the disc extending beyond the macular area and supplying at least half of the macula. In one eye a large cilioretinal artery was present on the nasal side. In another 35 eyes (4.7%) a medium sized cilioretinal artery extended up to the fovea and supplied some portion of the macular circulation. In 75 eyes (10.1%) cilioretinal arteries were small and did not participate in the macular circulation. In 14 of the 131 eyes more than one cilioretinal artery could be seen.

The distribution of refractive errors or C/D ratios in eyes with cilioretinal arteries did not differ from that for the whole series (Tables II and V). Table V shows that large cilioretinal arteries were common in eyes with minimal or no cupping (9/31).

and on the contrary infrequent in eyes with a large cup size (1.9%). This difference was however not statistically significant ( $\chi^2 = 1.312$ ,  $0.1 < P < 0.9$ ).

Cilioretinal arteries were commoner in children with low birth weight than in the whole group: one or two cilioretinal arteries were found in 8 of the 15 children studied (53.3%) ( $\chi^2 = 5.696$ ,  $0.01 < P < 0.05$ ). In 10 eyes the cilioretinal arteries were small, in two eyes of medium size. There was no correlation between C/D ratio and the presence of cilioretinal arteries in children with low birth weight.

#### Other findings on the optic discs

Optic disc drusen were diagnosed in three subjects; in one of them bilaterally. The child had bilateral cilioretinal arteries as well. One of the other two had a cilioretinal artery in the contralateral eye. In addition, drusen was suspected in 2 eyes. Myopic conus was present in 19 eyes, and a tilted disc was found in 1 eye.

### Discussion

According to Elschnig (1901) formation of the physiologic cup of the retina is modified by the extent of connective tissue during the phase of embryonic development in which the axons of the ganglionic cells approach the disc. If the connective tissue has been completely reabsorbed the axons might fill the posterior scleral foramen completely and the disc would therefore have no real physiologic cup.

In the present study minimal or no cupping was found in 23.5% of the children. Similar percentages have earlier been presented on adults by Ford & Sear (1961) 27.04% and by Hollows & McGuiness (1966) 23%. The similarity of these findings

Table 1  
C/D ratio in eyes with cilioretinal arteries

C/D	Eyes with cilioretinal arteries			Total	
	Large	Medium	Small	N	%
0.0-0.1	9	8	14	31	73
0.2-0.3	11	20	48	79	173
$\geq 0.4$	1	7	13	21	120
Total	21	35	75	131	100



Table VI

Survey of reported frequencies of chorioretinal arteries (CRA)

Author	No of subjects examined	% of eyes with CRA	% of subjects with CRA (unilateral/bilateral)
Jackson (1911)	500	19.1	29.6 (21.0/ 8.6)
Collier (1951)	1000	12.5	21.6 (18.2/ 4.3)
Lorentzen (1950)	172	15.0	26.0 (22.0/ 4.0)
Modeno (1974)	956	39.6	
Mustic & Lehmann (1976)	1000	32.1	49.5 (34.9/14.6)
Alouzas & Markakis (1978)	9000	26.5	42.6 (34.1/ 8.5)
Present series	350	17.7	27.3 (19.2/ 8.1)

as the finding that there was no significant difference in C/D ratio between the various age groups in this series indicate that there is no general enlargement in the cup size at school age or after except in eyes with moderate or high myopia. In the age group of 7-8 years a C/D ratio of 0.4 or more was noted in 14% of the cases which considerably exceeds the 2.77% frequency in infants presented by Richardson (1968). Thus it seems that some increase in cup size occurs during early childhood.

There is general agreement about the symmetry of the physiologic cupping between the two eyes (Snydacker 1964; Hollows & McGuiness 1966). In newborn infants marked asymmetry of disc cupping has been reported in 2.3% by Richardson (1968) and in 3% by Khodadoust et al (1968). In this series a difference of 0.2 or greater was fairly common in 5.2% of the cases which is between the cited figures in infants and those in adults presented by Armarly (8%) (1967).

Physiologic variation in refraction seems to have no significant effect on C/D ratio. Absent or minimal cups were equally common in myopic (23.8%) and hyperopic (22.7%) eyes. A high C/D ratio (0.4 or more) was however significantly more common in myopia of -2.0 D or more particularly if myopic conus was present, reflecting an enlargement of the posterior scleral foramen in axial myopia.

Pronounced cupping of the optic disc has been shown in children with low birth weight (Fledelius 1978). In the present study also the prevalence of large cups was greater in children with low (2500 g or less) birth weight than in the whole series but the difference was not statistically significant. In children with low birth weight chorioretinal arteries were also common but their presence did not correlate with the size of the cup.

The prevalence of chorioretinal arteries reported in the literature varies from 13 to

40% of the eyes (Table IV). The great variation might be explained by the unsimilar methods used. The highest figures of 39.6 and 39.1% of the eyes were estimated by means of fluorescein angiography (Sodeno 1974, Justice & Lehmann 1976). In the present series the frequency of cilioretinal arteries was estimated at 17.7% of the eyes approximating the figure of 15% defined ophthalmologically by Lorentzen (1970) in Denmark. Bilateral cilioretinal arteries were found in 10% of the subjects who had cilioretinal arteries which corresponds the estimates of Jackson (1911) and Justice & Lehmann (1976). Bouzas & Markakis (1978) reported bilateral cilioretinal arteries in 20% and Lorentzen (1970) and Collier (1957) more than 20%.

Mann (1928) attributed the development of cilioretinal arteries to the development of anastomoses of the posterior ciliary arteries with small branches from the hyaloid artery on the disc. The higher frequency of cilioretinal arteries in eyes with low birth weight support this concept. Thus theoretically factors involved in the reabsorption of the hyaloid arterial system could affect both on the presence of cilioretinal arteries and the development of physiologic cupping. In the present study we could not, however, find any correlation between the size of the cup and the presence of cilioretinal arteries.

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## OCULAR TRAUMA

### Observation in 105 patients

BY

ULFAR THORDARSON ARNI J RAGNARSSON  
and BJÖRN GUDBRANDSSON

A report on 105 cases of ocular trauma hospitalized in St Joseph's Hospital Reykjavik during a 12 year period is presented. Of these patients 94 were males and 11 females. Thirty nine patients were children and 65 were older than 20 years. Most common were occupational accidents (43 cases) and of these 29 were connected with industry. Other main causes were traffic accidents, sport and dangerous play by children. Of the total 113 cases, 10 had perforation of the bulb. All but nine underwent some kind of operation and enucleation was performed in 10 patients. Nearly two thirds of the patients were hospitalized within six hours of the accident and 94% within 96 h.

**Key words:** ocular trauma - causes of - age distribution of

The Department of Ophthalmology St Joseph's Hospital is the only ophthalmological clinic in Iceland and therefore serves all of the 230 000 inhabitants. Almost all cases with major ocular trauma occurring in the country are treated at the clinic.

This study aims at observing the causes of the ocular trauma, age and sex distribution of the patients and the kind of ocular trauma. We have also noted the outcome after treatment.

Received November 15, 1978

## Material and Methods

We selected randomly 100 cases with major ocular trauma treated at the St. Joseph's Hospital Reykjavík during the period 1960-1976. These cases ought to roughly represent all cases of ocular trauma occurring in the country during this period. The total number of patients treated at the clinic during this period was much larger. The study was done retrospectively by examining the hospital records and by telephoning those who could be reached (50 patients) and asking for the vision of the damaged eye.

## Results

## Age distribution

This is shown in Fig. 1. Children ( $\leq 15$  years) comprised 39 patients (37%) which is about the same ratio as in other studies made on this subject. Only six patients were older than 50 years.

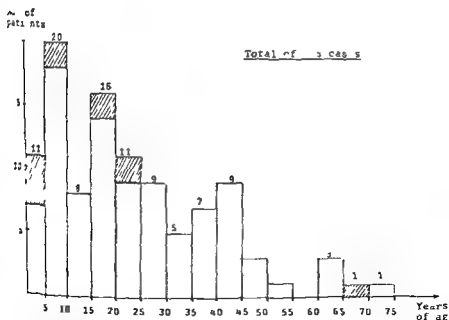


Fig. 1

The age incidence of the 100 cases of ocular trauma discussed in the article. Shaded area stand for females. Note the high incidence of children aged 15 years or less (39 cases) and that only six cases occurred after 50 years of age.

*Table I*  
The cause of ocular injuries in the 39 children  
discussed in the article

Cause of injury	Number of cases
Pointed objects (knives etc.)	6
Explosive materials (fireworks etc.)	4
Fall	4
Sport (winter sport & handball)	4
Arrows	3
Dart arrows	2
Catapulted missiles	2
Animals	2
Glass fragments	2
Miscellaneous	10
Total	39

*Table II*  
The activities of the 105 patients discussed in the article at the time of  
injury

Occupational injuries		43
I Industry	29	
Construction and building	14	
Automobile repair	7	
Engineering and cutlery	6	
Other	2	
II Fishing	3	
III Agriculture	4	
IV Miscellaneous	1	
Road accidents		3
Sport injuries		2
Domestic injuries		10
Miscellaneous (such as playing of children etc.)		7
Total		105

### Sex distribution

In this study 94 (90%) were males and 11 (10%) females. Eight of the females were younger than 20 years. See Fig. 1.

### Causes

When discussing causes of ocular trauma one can consider both the circumstances (place of the accident or the activities of the victim) and the object causing the trauma. For obvious reasons it is usual to classify the causes according to the former. See Table II.

**Occupational injuries** 43 of the total 105 cases (40%) are in this group, or 60% of the ocular trauma in adults. As would be expected most of these occurred in industry, or 49 cases, which comprises 28% of the total and 44% of the adults in the study. Perforation of the eye occurred in 17 of the 29 cases. Most commonly it was due to running wire on building sites (9), splints of metal or wood (8), screwdriver (6) and nails (9).

Industrial accidents are the most common cause of ocular trauma in Western Europe, and ocular trauma represents 3-5% of all industrial accidents according to Duke Elder's text book. Because of the sparsity of heavy industry in Iceland most of the accidents happen in construction and building and in light industry.

Only four cases could be traced to *agricultural work*, three of which were engaged in repairing fences.

Three cases could be traced to *fishing accidents*, which is surprisingly low when taking into account the large number of fishermen in Iceland.

**Traffic accidents** Eight cases with (which might be expected) severe trauma. At least two patients became totally blind in the eye, and four others had perforation of the bulb.

**Sport injuries** Only five cases, of which four were in children. Two cases connected with sking, 1 skating, 1 handball and 1 fishing. According to this, most eye injuries connected with sport are minor and therefore can be treated ambulatorily.

**Domestic injuries** Twenty cases had ocular injuries in the home, 10 children and 10 adults. Of the adults, four cases were connected with the use of alcohol (in the whole study 11 of 105 cases were connected with this). The most usual causes of injuries in the home were pointed objects, glass fragments and falls.

**Miscellaneous** This group comprised 29 cases, where the largest sub-group was outdoor playing of children.

Table III

The time interval between the time of ocular injury to the time of hospital admission with regard to the geographical location of the accident. Many of the patients were seen by a doctor before the admission and partially treated by him

Location of accident	Time interval from accident to admission		
	≤ 6 h	≤ 24 h	Total
Stor Reykjavík and Reykjanesskagi	45 (75%)	52 (81%)	97
Suðurland	4 (44%)	8 (88%)	9
Vesturland	7 (88%)	8 (100%)	8
Vestfirðir	2 (50%)	3 (75%)	4
Norðurland	3 (25%)	8 (61%)	11
Austurland	3 (97%)	8 (100%)	11
Total	64 (60%)	87 (84%)	101

### Children

Because of the large number of children in the study we want to deal with this group a little more closely (see Table I). The total number of children in the study was 39 of which 33 were boys and 6 were girls. Rather severe injuries are usual in this group with at least 6 cases who totally lost light perception in the injured eye and 20 other (60%) had perforation of the eye. Three had contusion of the eye ball and 1 developed traumatic cataract. Of the remainder most had a wound in the cornea, eyelid or the lacrimal duct.

### Time elapsing from accident to admission

This is of interest because the clinic serves the whole country which has a rather difficult communication system. Sixty four patients were hospitalized within the first six hours of the accident and 87 patients within 24 h. Generally the main cause of delay in this group was the long distance from the clinic but in the group which were hospitalized after 24 h the most common cause was that the severity of the eye injury was not recognized by the patient (see Table III).

### The nature of the eye injury

As evident in Table IV most of the injuries were serious otherwise they would have been treated ambulatorily. Of the 101 cases 70 patients had perforation of the eyeball.



Table IV

The types of injury in the 103 cases of ocular trauma. Concomitant perforation of the cornea and prolapse of the iris as the only injuries was described in 23 cases and of these 6 cases developed traumatic cataract

Type of injury (diagnosis)	As only injury or concomitant with other eye injuries	As the only eye injury
Ruptura bulbi	10	10
Perforatio corneae	19	19
Prolapsus iridis	2	0
Cataracta traumatica	10	0
Vulnus conjunctivae	13	0
Vulnus palpebrae	17	4
Ruptura canaliculi lacrimalis	8	0
Corpus alienum bulbi	9	4
Vulnus sclerae	9	1
Contusio bulbi	3	9
Vulnus musculi ocularis	3	0
Corpus alienum corneae	3	9
Prolapsus corporis vitreum	4	0
Expulsio lentis	3	0
Trauma orbitae	2	0
Prolapsus uvulae	9	0
Prolapsus corpora ciliare	1	0
Iridocyclitis traumatica	1	0
Endophthalmitis	1	0
Ophthalmia sympathetica	1	0
Ablatio retinae	1	0

#### Treatment

The purpose of this paper is not to make a detailed discussion on the treatment of the ocular traumas. All but nine of the 103 patients had to undergo some kind of operation. Enucleation had to be performed in 10 patients. Most patients with perforation of the bulb were treated with antibiotics and cortico-steroids.

#### Revision on the affected eye after treatment

We tried to call all the patients by telephone and managed to reach 50 out of the 103. They were asked about the sight on the involved eye. The results are shown in Table V.

Table 1

The subjective valuation of sight by the 50 patients contacted for follow-up (regardless of the use of eyeglasses or lenses)

Total	Blind	Sees light	Sees outlines	Can read	Sees well in the distance and for driving	Percent seen
50	6	4	5	1	0	52

Of these 50 patients 32 considered that they had perfect vision on the left eye, six patients had complete blindness of the eye and nine patients had very poor vision. Of the group which we did not reach eight patients had undergone enucleation. Therefore at least 14 patients of the 105 became blind in the right eye. Of these 14 patients six were children and most of the rest were under 50 years of age with mean age of 22 years.

## Discussion

The results speak for themselves and need no long discussion. In general we can say that the causes of the ocular trauma in our study are much the same as in previous studies on the same topic. The study stresses the need for prophylactic measures to prevent eye injuries both occupational (especially within industry) and recreational of children.

The age distribution shows that ocular trauma occurs mainly in young people and as previously known it is the most common cause of unilateral blindness in children and young adults. Females comprise only 10% of the total number of patients and people over 50 years of age are seldom involved.

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## AUTOMATIC PERIMETRY IN A POPULATION SURVEY

BY

BO BENGTSSON and C. E. T. KRAKAU

Automatic perimetry was performed in 2998 eyes of 1411 subjects comprising 8% of all persons born 1907–1921 and resident in a certain small area. Unreliable or abnormal tests were repeated. The average number of tests per person was 2.25. About 90% of all tests in normal eyes were performed in less than 3 min. The screening was considered negative in 2887 eyes, incomplete in 10 eyes and positive in 91 eyes (3%). Eighteen of the positives were previously unknown glaucomatous defects. There was only one unexplained false positive. We concluded that the method is quick, sensitive, specific and dependable. The apparatus is simple to manage and cheap to run.

*Key word:* defined population – visual field defects – automatic perimetry – glaucoma.

During the last few years an automatic perimeter has been developed and tested at the Department of Experimental Ophthalmology in Lund (Heijl & Krakau 1975a,b, Krakau 1978). A great deal of clinical experience was gained i.e. the instrument was used on a material of glaucomas and glaucoma suspects (Heijl 1976). A trial in a population survey of the logic for detection of glaucomatous visual field defects was considered appropriate.

A preliminary report comprising about one third of the present material was given at the Nara Symposium (Bengtsson & Krakau 1979).

Received March 5, 1979

## Material

All persons born 1907–1921 and resident in the district provided for by the Community Care Centre were listed in December 1976. The list was arranged according to the residential addresses and kept up to date by means of reports from the County Council on removals and deaths. Following this list the inhabitants were contacted in rotation during 1977 and 1978 and underwent repeated ophthalmological examinations. Attempts at persuasion were made and only persons able and willing to attend within a few weeks were included in the survey.

One person known to be blind and 24 patients subjected to anti-glaucoma therapy were not invited. There were only five cases with field defects (three congenital and four secondary glaucomas) in this group.

Out of 1938 invited persons 1511 (78%) took part in the survey. The rate of attendance was largely independent of age and sex.

Six persons had lost one eye. 16 were blind in one eye and 2 were unable to look straight ahead with one eye. Automatic perimetry was attempted however in each one of the 2998 seeing and mobile eyes of the 1511 subjects in the survey.

## Methods

Sphygmomanometric measurement of the systemic blood pressure, determination of the visual acuity, subjective refraction, automatic perimetry, fundus photography, indirect ophthalmoscopy, slit lamp examination and Goldmann perimetry were attempted in every case. Perimetry and photography were conducted by two alternating assistants – ophthalmoscopy, slit lamp examination and perimetry by one of the authors (BB). Perimetric data handling was entirely automatic and the data were immediately recorded on special forms. Transfer to magnetic tape and further processing were performed at the Computer Centre in Lund.

In the present report we have confined ourselves to the results of automatic perimetry.

**Perimeter.** The perimeter described by Heijl & Krakau (1973) was used. Six points are fixed in a pattern of concentric circles at 5, 10 and 15 degrees eccentricity. At 20 degrees there are eight points placed as shown in Fig. 1. At each point a LED (light emitting diode) can be made to emit light at anyone of 16 levels. The relation of luminance between two consecutive levels is 1.2. One further light spot is projected on to the perimeter screen, adjusted so as to fall in the blind spot area and lit at random intervals on an average 1/9 of all trials. If the patient keeps

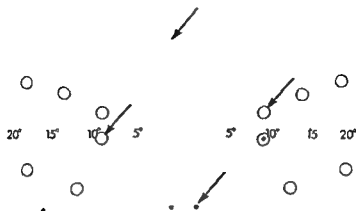
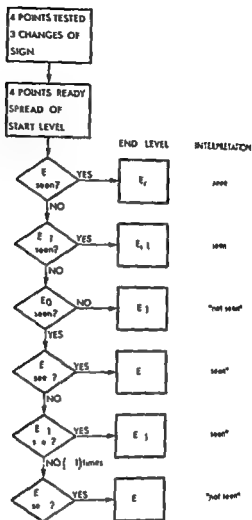


Fig 1

**Location of test points** The threshold is first determined at the four places pointed at by arrows. When one or two of the points (●) and eventually one or two of the points (○) are not seen the blind spot is denoted normal. (The right group refers to the right eye and vice versa.) The central point is a red fixation light.

his fixation it cannot be seen. The patient signals that he has perceived a light by pressing a button.

**Test time** The test procedure is similar to the programmes for glaucoma screening in manual perimetry as used by Armaly, Drance and others. However, the points tested are chosen in random order in the computerized version. Minor changes only have been made in the programme used by Heijl (1976). The threshold is at first determined at four points on the 10 degree circle (Fig. 1). If at a certain point, a target is seen, next time it will be shown on a one step fainter level and vice versa. The target perceived is accepted as the threshold when the process has changed its direction three times. A *supraliminal* test value is calculated from the thresholds of the first four points and used as a starting level for the remaining test points. If at any point this level is seen, this point is not further tested; if not, the intensity is increased by one step. If this level is seen, this point is again not tested; if not seen, the LED is lit at its very highest intensity level. If still not seen it is not tested again. If the highest intensity level is seen, the process returns to the *supraliminal* starting level, passes step by step to higher intensities as long as the light is not seen, and ends as soon as the light is seen again (Fig. 2).



*Fig 2*

Flow diagram showing the course of testing one eye at F with level F highest intensity level

**Test conditions** As a rule the blind spot stimuli for fixation control were directed towards a point about 2 cm temporally and inferior to the test chord 15 sec, away from the fixation light. An individual adjustment was resorted to only when an anomalous position of the blind spot was suspected. Only spherical glasses were used to correct for near vision and no special steps were taken to secure dark adaptation. The instructions to the subjects (to fixate the red light and to press the button whenever a white light was seen) were kept as few and simple as possible. The assistant remained in the room for a while but left it as soon as the first successful cooperation was satisfactory.

**Presentation of results** The results were presented by means of a keyboard printer (Silent 700 TEXAS Instruments). The printout of one field chart required less than 1 min.

**Interpretation.** A test point was considered *seen* if it had ended on a level not more than one step lower than the initial one (Fig. 2).

Up to four points not seen in the blind spot area (Fig. 1) were accepted as a *normal blind spot*. If the blind spot was missed and in addition the blind spot check light seen more than five times the test was regarded as *unreliable*.

A reliable test was considered *normal* if all points outside the normal blind spot were seen (otherwise *abnormal*). If a reliable test was normal the eye was classed as *positive*.

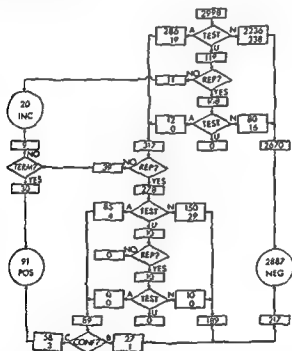


Fig. 3

Flow diagram used when classifying the field screening of an eye as positive or negative. The number of eyes following a certain course is given in the cartouches. The upper number refers to tests in which the blind spot was found – the lower to tests in which it was missed.

REP = Repeition of test<sup>2</sup>  
 TERM = Termination of screenings<sup>2</sup>  
 CONF = Confirmation of defects  
 INC = Incomplete screenings  
 NEG = Negative screenings  
 POS = Positive screenings

L = Unreliable tests  
 N = Normal tests  
 A = Abnormal tests  
 B = Unconfirmed defects  
 C = Confirmed defects

When a reliable abnormal test was obtained in an eye with a known field defect or in which a defect could be attributed to an ophthalmoscopic lesion, the screening was terminated and considered positive. With this exception the screening of one eye was not considered complete unless all unreliable or abnormal tests were repeated.

If the first reliable test was abnormal a proposed defect was assumed. If such a defect was followed by a similar one in the same eye the points outside the second blind spot were compared. If at least one of those points was not seen in the second test showed a confirmed defect and the screening was considered positive. If a proposed defect was not reproduced at any point in the second test, the screening was considered negative.

The final classification of screenings as incomplete, positive or negative followed rules so strict that the computer was entrusted with it (Fig. 3). The operator had to initiate a retest but even this task could have been left to the computer. An ophthalmologist had to decide whether a termination of the screening was warranted (and to reclassify terminated screenings from incomplete to positive).

As indicated in the preceding paragraph even the immediate interpretation of each test, ordering a retest according to the strategy adopted etc. could have been left to the computer. In the course of the survey a major part of the software necessary for a complete automation was developed for the purposes of supervision and arranging the material. For a start, however, we had to resort to a semi-automatic procedure involving a presentation of the results in a cruder form and a human element — which was retained throughout the entire study.

## Results

The background illumination was in nine eyes with nuclear cataract 0.1 cd/m<sup>2</sup> and in others 1 cd/m<sup>2</sup>. There was no difference in the threshold reached between the first (right) and the second (left) eye. Thus there was no indication of dark adaptation.

The time needed for one test was less than 3 min in 90% of all normal eyes. The average number of tests per person was 2.25.

Eleven unreliable and 9 abnormal tests were not repeated — because of technical subjects or because the subject was not motivated to continue (9 subjects). The field screening was completed in 2978 eyes — 2887 negative and 91 (3%) positive (Fig. 3).

The positive field screenings are summarized in Table 1.

Six known (nonglaucomatous) field defects were all spotted by the automatic parameter. We also soon learned to expect diffuse centroretinal defects or ring field defects in highly myopic eyes with posterior staphyloma and/or peripapillary atrophy.



Table I  
Positive screenings

	No of cases n = 1505	No of eyes n = 2978
Known (nonglaucomatous)	6	6
Expected (coecentral)	18	23
Unknown and unexpected	38	46
False positives	14	16
Total	76	91

From our point of view the actual yield of the field screening was 46 *unknown and unexpected defects* — 18 glaucomatous and 28 "others" — described in Table II. They were all reproduced and also verified by Goldmann perimetry, if not by ophthalmoscopy. Their extension was in general adequately mapped out by the automatic field chart.

Table II  
Unknown and unexpected visual field defects

	No of cases	No of eyes
A Glaucomatous		
1 Arcuate	6	7
2 Arcuate and circumscribed	1	2
3 Circumscribed	8	9
Total	15	18
B Others		
1 Of unknown etiology*	"	"
2 Circumscribed paracentral — corresponding with various small but obvious fundus lesions	18	21
3 Homonymous paracentral	2	4
4 Bitemporal	1	1*
Total	23	28

One defect had disappeared at follow up — the other turned out to be part of a homonymous one.

Automatic perimetry was not attempted in the left eye in which the visual acuity was  $\approx 60$ .

The 18 glaucomatous defects were found in eyes with pathological changes of the disc. All patients with glaucomas are of course followed up and a detailed account of the characteristics associated with glaucomatous field defects in the present material is planned.

The 28 others were caused by a wide variety of disorders among which occlusions of small retinal veins (5 cases) and scars in retina and choroid (11) were most frequent. Apart from the glaucomas only two cases with vascular defects (one chiasma compression and one retinal detachment) needed medical care.

The field screening was considered to be false positive in 14 cases (16 eyes). Two subjects could not be prevented from overfixating. One deaf person was an excellent lip reader and therefore by mistake left without adequate instructions in the dark room. In six cases extraocular causes such as ptosis, blepharitis, smudged glasses and matted (super)cilia partly blocking the sight of the test were overlooked and therefore not eliminated. Repeated telephone calls disturbed one individual and another suffered from migraine. To sum up, false positives could usually be given an adequate – though trivial – explanation. Only one subject had to be left unexplained and not verified by kinetic perimetry.

The blind spot was "missed" in 12% of all reliable tests. This rate was higher if the fixation was good (i.e. the check light seen  $\leq 2$  times) and still less than 1% when the fixation check light was seen three to five times.

The results in geographical subdivisions of the material were alike and the achievements reported at the beginning of the survey were well maintained or improved – at the end. The rates of unreliable tests, false abnormal tests and missed blind spots in repeated tests were similar to those in initial tests. False abnormal tests were twice as common in men as in women. The frequencies of missed blind spots and false abnormal tests increased in the older men and decreased in the older women. All types of true defects were more prevalent in the older half of the population.

## Discussion

When first recognizable glaucomatous field defects are small circular central deep scotomas which may appear anywhere in the central field (Aulhorn & Harter 1967). Any attempt to detect progressive defects smaller than the blind spot is obviously be unprofitable since perimetry cannot be performed accurately. The perimeter treats the blind spot area in exactly the same way as the rest of the paracentral field. The fact that the probability of detecting the blind spot by automatic perimetry is as high as about 0.9 should therefore mean that the sensitivity

amply fulfils any reasonable demands on *sensitivity*. Surely the detection of 46 unknown and unexpected defects does not contradict this conclusion.

The rate of *verified defects not warranting medical care* (2%) was greater than might be desired but of course a natural and inevitable consequence of the high sensitivity.

Our main concern was however *specificity* since a high rate of false positives might easily become a major obstacle. We were therefore much relieved to find that after retesting very few unexplained false positives remained.

We conclude that the procedure of automatic perimetry in the form applied is quick sensitive specific and dependable.

### Acknowledgment

This work was supported by the Swedish Medical Research Council (proj No B79-04\ 5009-09A) which is gratefully acknowledged.

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## JUDICIA DE NOVIS LIBRIS

*J. Beres: The Diagnostic Limitations of Computerized Axial Tomography. Springer-Verlag, Berlin Heidelberg New York 1978. 173 figures, 2 tables, 91 pages, D.M. 44, US dollars 27.-*

The excellent method of computed tomography (CT scan) has completely altered the face of modern neuroradiology. Accordingly the first wave of clinical interest has been very enthusiastic.

Of course there must be limitations to CT and it is nice to learn that CT has now established that it is also open to criticism.

This is reflected by the present congress report (Europ. Soc. Neurosurgery, September 1976) in which the contributions focus not only on the established merits of CT but also on the diagnostic limitations of the method. A sound approach indeed leads to a better differential diagnosis by CT — and leaving credit to the established methods (CT and other) with the most suitable combination to be chosen by the clinician according to the demands of the single case.

The 24 reports are grouped into five sections. The diagnostic limitations of CT in 1) cerebral tumours, 2) diseases of the orbit and of the skull base and the face, 3) intracranial infarcts, oedema and subdural haematomas. Section 4) deals with CT and other neuroradiological techniques and 5) with accuracy and further prospects of CT.

In part 2) the article on orbital problems (by Moseley et al.) including also the new role of special interest to ophthalmologists.

Otherwise the book primarily addresses itself to neuroradiologists. It is interesting especially for those who have recently started working with CT but are not yet experienced with the possibilities of the technique.

H. J. J.

*A. Schuchardt and A. Schumacher: Fortschritte der Kiefer- und Gesichtschirurgie 1978. Buch Band 111. Plastische Chirurgie im Mund, Kiefer und Gesicht. Georg Thieme Verlag Stuttgart 1979. 100 pages, 148 illustrations, Price D.M. 14,-*

This yearbook contains a series of brief discussions of various aspects of reconstructive surgery. Most of the techniques presented would seem not to be relevant to an ophthalmologist and only one paper can be said to fall within the field of traditional ophthalmic surgery. This paper describes correction for blepharochalasis.

Although therefore one has to say that this book is of only limited use to an ophthalmologist it could as well be represented as a brief, well illustrated and up-to-date survey of what neighbouring surgical specialties can offer the multi-traumatized and deformed patient. With this background it can be recommended to surgeons and ophthalmologists.

**Farré A de Roock** *Electrodiagnosis Toxic Agents and Vision* 1th ISCEV Symposium Ghent 1977 Junk The Hague Boston London 1978 343 pages Price Dutch Guilders 110.-

This book consists of 34 papers delivered at the 10th symposium of the International Society of Clinical Electrophysiology of Vision which was devoted to electrophysiological findings and diagnosis in connexion with toxic or other damaging effects on the retina the pigment epithelium the visual pathways and the visual cortex.

Among the substances showing or tested for effects on ERG EOG or VEP components are chloroquine thioridazine gentamicin diphenylhydantoin barbiturates urethane alkane ethanol methanol zinc iron copper (metallosis bulbi) CABA taurine gangliosides sodium iodate and neurotoxins. Other damaging effects included photocoagulation trauma and ischaemic retinopathy.

Toxicology of drugs and other substances is becoming increasingly important in clinical practice as well as in research. This book which gives a rather good picture of the field of electrophthalmology may be warmly recommended to ophthalmologists and electrophysiologists interested in toxic effects on the visual system.

*S. F. A. M.*

**Junker Friedel** *Kontaktlinsen Praxis einer optimalen Anpassung* Urban & Schwarzenberg München Wien Baltimore 1978 140 pages 90 figures Price DM 34.-

Nearly two per cent of the population in Germany wear contact lenses training in contact lens fitting is an educational routine in major eye departments. This book addresses itself to the beginner who wants to learn practical fitting techniques. Its synopsized style does not yield much space for theoretical considerations but most of the text is devoted to practical means. Hard and soft lenses of classical or more recent composition (CAB sil one polycarbonate) are discussed as well as different designs of toric lenses. A tabular summary of the commercially available lens types provided with the manufacturer's fitting instructions and a list of the major brand contact lens care products with a description of their chemical constitution provide useful information.

The author treats his subject in a reporting form which now and then leaves the reader with a personal attitude based upon the author's practical experiences.

However the book can be recommended either as an impetus to start or as a basic textbook for those ophthalmologists interested in the fine art of fitting contact lenses.

*S. D. M.*

**Klaus Heilmann** *Therapeutic Systems pattern specific drug delivery: Concept and development* Georg Thieme Verlag Stuttgart 1978

The book deals with the principles of controlled or monitored drug delivery and the remarkable results Alza Corporation Palo Alto California, USA have achieved in this field.

In the first part of the book, the author defines the biopharmaceutical principles forming the basis of the development of new therapeutic systems i.e. new ways of administering drugs such as continuous drug release at a certain rate per time unit. He discusses the limits of conventional forms of drug administration and gives a short survey of possible membranes

and energy sources for new therapeutic systems. In the last part of the book, new therapeutic systems now in use in medicine and as experimental models are described. It includes Therapeutic Systems for systemic use, Therapeutic Systems for local use, osmotic minipump and a system with a so-called Closed Circuit (coupled to an artificial beta cell).

For ophthalmologists the most interesting part is that describing the Ocusert, a new therapeutic system. Here a broad and for the practicing ophthalmologist unique description is given beginning with the old Greeks. The book is a substantial and gives a good introduction and understanding of pharmacokinetics and technology of delivery. This is of importance not only for all those using the Ocusert delivery system but also medical practitioners. Knowledge and technology in these fields is increasing rapidly and one can expect to see a number of therapeutic systems developed and put into use in medicine in the near future.

The book is easy to read with a number of good and instructive figures and is suitable for students and medical practitioners alike.

*Dirk Vroom*

*Augenärztliche Fortbildung. Jahreskurse für die praktische Augenheilkunde. Band 6. Teil 1. 1976. Herausgeber: Hanns Jürgen Metze. Urban & Schwarzenberg.*

This book is the third in a series of four books designed for the postgraduate training of ophthalmologists.

Three different topics are treated in the book: traumatic retinal detachment (L. von C. Artner), the neuro-ophthalmology of eye muscle palsies (the differential diagnosis of W. Alfred Huber) together with ophthalmoscopy (E. Kleiberger).

The book is easily read and is well illustrated but presents nothing new. The material without difficulty be found in the standard textbooks as Duke Elder or otherwise. The book is therefore hardly of interest to Scandinavian readers.

*J. J. Jørgensen*

*Glaucoma. Conceptions of a disease. Pathogenesis. Diagnosis. Therapy. (Series: Themed Books. Verlag Stuttgart. Distribution for USA by W. B. Saunders Company.) 1976. Igaku Shoin Ltd. Tokyo. 434 pages. 92 figures. 93 tables. 1 colour plate. Price \$128.-*

As indicated by the title the purpose of the book is to give a comprehensive survey of the glaucomas.

The book is divided into eight sections: 1) Glaucoma. Conceptions. 2) Pathogenesis. 3) Physiology and pathology. 4) Glaucoma damage. 5) Methods of examination. 6) Pathology. 7) Management of the glaucomas. 8) Surgical techniques. 9) The classification and synthesis.

Thirty-one acknowledged experts in the different fields of glaucoma from a total of 19 countries have succeeded in giving an inspiring description of the present day picture of glaucoma. After a somewhat disappointing period with focus on the medical treatment, new advances are to be expected to be made in the two other areas of the clinical field, namely problems concerning damage to the optic nerve head and disturbances in the visual field.

For the reader who wishes to further supplement his knowledge of the glaucoma the book adds with 39 pages of references which satisfactorily cover the more recent publications up to the beginning of 1977

The book is of value to the ophthalmologist who wishes to have an up-to-date account concerning the problems of glaucoma, which continues to give rise to a most extensive stream of information.

*Knut Vøskov*

Berufsverband der Augenärzte Deutschlands Arbeitskreis Kontaktlinsen. Einführungs und Fortbildungsvorträge der Arbeitskreistagungen, Wiesbaden 1966-1973 Edited by M. Freigang 1976 (Nürnberg)

The book comprises a collection of reports of approximately 60 lectures given in Wiesbaden in the years between 1966 and 1973. The book contains many topics with the main stress laid on the technique of fitting contact lenses, the method of using contact lenses in keratoconus, astigmatism and aphakia, as well as the fitting of contact lenses in children.

The book also contains several sections on the physiological and pathological changes seen with the use of contact lenses.

The book offers nothing new and can hardly be said to be of great interest. It cannot replace the standard textbooks on contact lenses of which there is now a good selection.

*J. A. Fahmy*

E. J. Klein: Die Schilddrüse. Diagnostik und Therapie ihrer Krankheiten. 2nd ed. Springer Verlag, Berlin, Heidelberg, New York, 1978. 904 pages, 61 ill. Price DM 68— or US dollars 34—

The present edition is a completely revised up-to-date successor to the 10-year-old first edition with stress on the medical endocrinological problems.

The diagnosis of endocrine ophthalmopathy can be a considerable problem for the ophthalmologist. The most recent hormonal methods of examination are discussed. It is emphasised that a combination of several tests is necessary. The metabolism can as is well known be normal or even reduced in endocrine exophthalmus.  $T_3$  will be relatively raised in relation to  $T_4$ . The radio-iodine uptake curve in the thyroid gland is raised. Suppression with  $T_3$  will be negative. Thyrotropine releasing hormone will not increase the thyrotropine blood level.

These tests and their limitations are discussed on the basis of about 4000 patients with all types of thyroid disorders of these 1500 had an ophthalmopathy.

During treatment great importance is attached to preventing hypophyseal induced exophthalmus (antithyroid treatment combine with steroid and thyroid hormone). The difficulty in diagnosis and therapy is demonstrated.

Positive autoimmune reaction, L.A.T.S., D-thyroxine, roentgen treatment of the apex of the orbit, etc. are considered.

The book is well written and well illustrated with photographs of patients and instructive diagrams. It may serve to further the co-operation between the intern medical specialist and the ophthalmologist.

*M. S. Vorn*

# *Third International Conference on Myopia*

The International Society for Myopia Research announces the Third International Conference on Myopia to be held in Copenhagen, Denmark, 24th to 29th August 1990.

Open to members of the Society and others actively involved in myopia research.

Official language: English

Abstracts should be submitted before March 1st, 1990

For further information and abstract sheets, contact: Third International Conference on Myopia, University Eye Clinic, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø.

Persons interested in joining the Society should contact the secretary, Francis A. J. G.

Ph.D., International Society for Myopia Research, P.O. Box 9099, C.S. Post Mall, Washington

99163, U.S.A.





(1885) This was subsequently confirmed by the classic investigations of von Wald & Buschke (1944) Buschke (1949) Heydenreich (1954) and Huxley & Huxley (1960) Experimental wound healing as described by several authors previously involves extensive wounds and various complicating factors rather than the detailed movement of the epithelium

The exact morphological presentation of the possible cell movements has now been limited by the resolution of the light microscope The use of the scanning electron microscope makes it possible to examine the cell surface at higher magnification Pfister (1975) has for the first time used this technique to examine the healing of purely epithelial wounds

The purpose of our study was to demonstrate in detail the behaviour of epithelial cells and their morphological changes during the repair phase of the healing of deep corneal wounds

### Materials and Methods

Thirty-one fully grown rabbits with healthy eyes were used. The animals were anaesthetised with an intramuscular injection of ketamin (ketanest® Parke Davis 25 mg/kg body weight) and xylazin (Rompun® Bayer 0.2 mg/kg body weight)

*Group A (10 rabbits)* Using a scalpel with the aid of a surgical microscope a superficial but non penetrating cut of approximately 5 mm length and 0.4 mm depth was made The corneas were then examined after 0 1 3 6 and 12 h

*Group B (21 rabbits)* With the aid of a surgical microscope a central lesion 4 mm in depth was made using a 2.5 mm trephine The epithelium and stroma within the circular lesion were removed with a hockey knife The corneas were examined after 0 6 15 24 48 96 and 120 h The animals were killed with an intravenous injection of 20% N (2 (m Methoxyphenyl)-2 aethyl-butyl-4 (1) 4 (1) butyramid + 5% 4 4 Methylene bis (cyclohexyl trimethyl ammonium salts) 0.5 4 Butylaminobenzol 2-dimethylamino-aethanolhydrochlorid (T 61® Hoechst 1 ml) and the corneas were immediately irrigated for 5 min with 4% glutaraldehyde in 1/15 M Sørensen's phosphate buffer (pH 7.2) before the corneas were removed using a 9 mm trephine They were then treated according to Pfister (1975) i.e. washing with 20% acetylcystein (Mucolyticum Lappe® Lappe) for 10 min, washing again for 10 min then washed in 20% acetylcystein and finally fixed for 24 h After washing the preparation in Sørensen's phosphate buffer for 1 h they were further fixed in osmium tetroxide in phosphate buffer and dehydrated in increasing concentrations of acetone The dehydration of all preparations was achieved using critical point drying All preparations were coated with a 20 nm gold film and examined in a Stereoscan 600 (Cambridge)

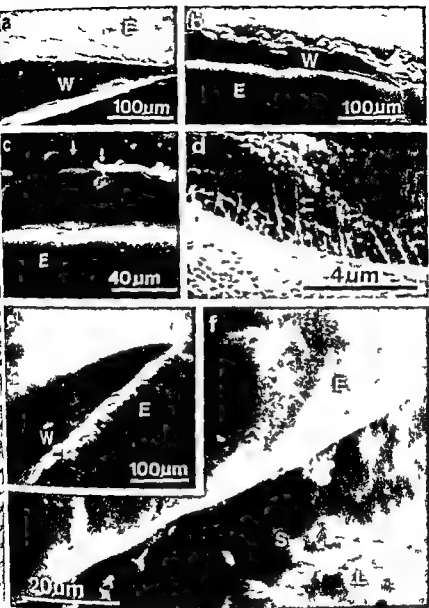


Fig 1

- a) Zero-time after linear wounding. The wound opening (W) is wide and the wound edges are retracted. Epithelium (E) (240 $\times$ )
- b) 1 h after linear wounding. Detached epithelial cells in the wide wound (W) epithelium (E) (240 $\times$ )
- c) 1 h after linear wounding. Detached epithelial cells (arrows) the wound edge is covered with sliding epithelium (E) (480 $\times$ )
- d) 1 h after linear wounding. Filopodia (arrows) extend out toward the wound (720 $\times$ )
- e) 3 h after linear wounding. The wound opening (W) is wider and the edges are covered with epithelium (E) (240 $\times$ )
- f) 3 h after linear wounding. Sliding epithelium (E) has covered the wound edge. Leukocytes (L) invade into the stroma (S) (1450 $\times$ )

## Results

## Group A

*Zero time after corneal wounding (4 eyes)* There is a clear retraction of the wound edges and the whole wound gapes open (Fig. 1a). At about the same time the epithelial cells round off and the stroma fibers appear to shrink correspondingly.

*1 h after corneal wounding (4 eyes)* At the edge of the wound are detached epithelial cells which were damaged during the wounding and are now shed (Fig. 1b). Superficial stroma slide under the damaged cells over the rounded wound edge (Fig. 1c). These cells possess densely accumulated microvilli and microplicae and extend into the wound area (Fig. 1d).

*3 h after corneal wounding (4 eyes)* As before a gaping wound can be seen. The superficial epithelial cells are however already spread over a large part of the stroma (Fig. 1e). They display a regular complement of microvilli and on their surfaces. For the first time leucocytes are now found in the wound (Fig. 1f).

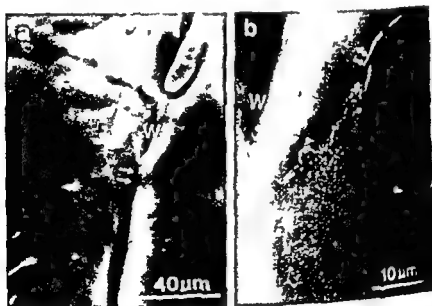
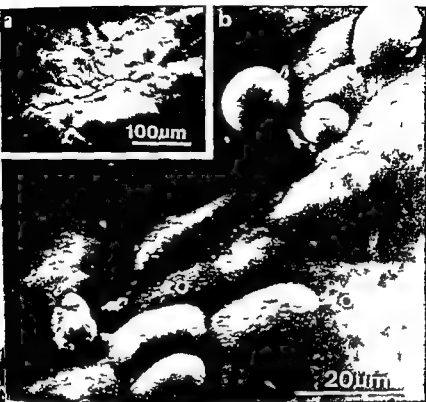


Fig. 1

6 h after linear wounding

- a) In some areas the wound edges have already adhered (arrow) (180 $\times$ )  
 b) The wound edge (W) is covered with sliding epithelium, the epithelial cells show interdigitations (arrows) (1800 $\times$ )



**Fig 3**

**15 h after linear wounding**

Original wound (arrows) is filled with epithelial cells (142x)

Epithelial cells in the original wound area are interdigitated with each other and appear compressed and rounded off (1400x)

**15 h after corneal wounding (4 eyes)** The wound slit is narrower. In isolated areas the wound edges have already adhered (Fig 2a). The superficial cells are also found deep within the wound cleft. Outside the actual area of the wound many dark epithelial cells are apparent, some of which have no regular microstructure. These cells interact loosely with each other (Fig 2b).

**25 h after corneal wounding (4 eyes)** The wound slit has closed and can only be recognised by the epithelial structure (Fig 3a). The epithelial cells in this area appear compressed, rounded off and partially overlap one another (Fig 3b). They also have a dense microstructure on their surface. This indicates an excessive proliferation. In other regions in the previously damaged area very narrow epithelial cells are seen whose elongated axis lies parallel to the wound slit.

## Group B

*Zero time after deep corneal lesion (6 eyes)* The whole wound edge appears retracted (Fig. 4a). Detached epithelial cells lie at the wound edge while the underlying lamellae and open tissue lesion remain exposed (Fig. 4b).

*6 h after deep corneal lesion (6 eyes)* In the bed of wound large numbers of leucocytes are found (Fig. 4c). The original vertical edge of the wound is clearly visible (Fig. 4d). The superficial layer of epithelial cells which can be traced from the undamaged epithelium into the bed of the wound has slid over the wound edge.

*15 h after deep corneal lesion (6 eyes)* Striking in this phase are the numerous club-like cell processes which extend themselves from the superficial cells on a virtual migration front out over the stroma (Fig. 5a). They are 1–3  $\mu$ m long and 5–8  $\mu$ m wide. These processes possess numerous short microvilli (Fig. 5b). For the first time the basal cells can also be seen. These spread up from under the rounded and superficial cell covered wound edge.

*24 h after deep corneal lesion (6 eyes)* About one half of the original bed of the wound is epithelised. Different forms of migrating epithelial cells are now found (Fig. 6). Sometimes these cells possess smooth cell edges which nestle to the corneal stroma. In other cases the cell walls of the migrating cells show a coral-like appearance. This impression originates in this case from the fact the cell projections also grow perpendicularly to the area of the wound.

*48 h after deep corneal lesion (6 eyes)* A nearly complete epithelisation is reduced to a small central region of approximately 250  $\mu$ m diameter (Fig. 7a). The wound edge is still clearly visible. Leucocytes are no longer seen in the wound region. The epithelial projections which have slid from the original wound edge to the centre interdigitate with one another (Fig. 7b). Further migration takes place from all sides in impression of extensive epithelisation sometimes given. Cells project out over the surface (Fig. 7c).

## Fig. 4

a) Zero time after deep corneal lesion. Retracted wound edge.

(W) if stroma corneae (72  $\times$ ).

b) Zero-time after deep corneal lesion. Detached epithelial cells.

(72  $\times$ ).

c and d) 6 h after deep corneal lesion. The wound bed.

Leucocytes (L) at wound edge.

(Fig. 4d) 400  $\times$ .



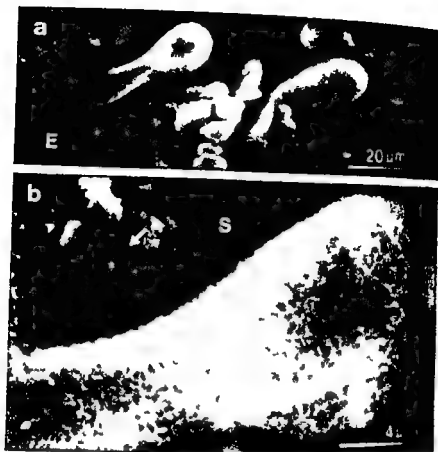


Fig 5

1 d after deep corneal lesion

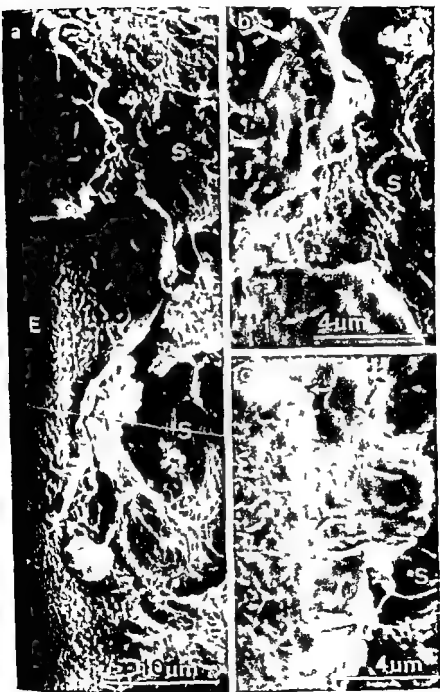
- a) Voluminous cell processes extend from superficial epithelial cells (E) over the cornea (S) Leucocytes (L) (14,500x)  
 b) Cell process with short microvilli basal epithelial cell (art m) near cornea (S) (72,500x)

Fig 6

24 h after deep corneal lesion

- a) Sliding epithelium (E) with different forms of microvilli cells near cornea (S) (20,000x)  
 b) Flat cell with a microvillous free zone (L) and cell with acetal like appearance (art m)  
 c) Ruffles of cell margin sometimes perpendicular to the cell surface (art m)







96 h after deep corneal lesion (6 eyes) The original region of the wound of 2.5 mm diameter is completely covered with a layer of epithelium (Fig 7c) The epithelial cells of this region appear largely dark and the contours of the cell nuclei can be seen (Fig 7d) In some places disruption of the cell association occurs

170 h after deep corneal lesion (6 eyes) The original lesion is still recognisable as a depression The surface relief of the epithelial cells has reached an almost normal condition

## DISCUSSION

The sliding of the epithelial cells is a common feature seen in wound healing of the cornea. This investigation has been able to demonstrate morphological details relating to the sliding cells. Initially the whole wound edge retracts slightly so that the superficial cells of the corneal epithelium can migrate over the whole wound within the first 6 h after wounding. When the stroma has also been damaged the cells must slide not only over the open cut in the epithelium but also over the left in the stroma. The closure of the wound edge perpendicular to the surface is achieved through additional enlargement of the surface of these cells which under normal conditions are very flat. This sealing of the wound periphery protects the subsequently regenerating basal cells from noxious substances. The cells appear to draw their potential for expanding their surface from the otherwise densely accumulated microprocesses since during the migratory phase these cell processes are sparser in the covering of large wound areas than in that of a narrow cut. After approximately 1.5 h a transition phase which is conspicuous in extended wounds is reached. In some areas the movement is still led by superficial cells while in other regions of the wound edge basal cells are already moving out from under the superficial cells.

After 24 h the epithelisation of the heavily damaged stroma is finally taken over by the basal cells which are then followed by further cell layers. The sliding cells adapt their shape to that of the tissue cavities. Because of the damage to the basement membrane the adhesion to the epithelium is poor and only after 96 h can

Fig 7

- 48 h after deep corneal lesion. The area of the original defect is nearly completely epithelised up to a small residual defect. The original wound edge (W) can be noted (145x).
- 48 h after deep corneal lesion. Central area of the residual defect. Epithelial cells stroma (S) (2900x).
- 96 h after deep corneal lesion. The original wound is completely covered with epithelium. Original wound edge (W) (145x).
- 96 h after deep corneal lesion. The surface cells are predominantly large and dark with visible nuclei (N) (190x).

the epithelium close a lesion of 2.5 mm diameter. The superficial wound (the epithelial cells is very different from its normal state emphasizing the fact that epithelialised wounds are very vulnerable. The intercellular connections and the microprocesses of the plasma membrane are only poorly defined. The irregularities of the whole epithelium cause the precorneal film to adhere to it and the appearance of dehydration in some areas is possible. The very dark cells with visible nuclei are dehydrated cells. Disruption of the epithelium is, however, any time up to this point. The surface relief of the sliding epithelium has been approached that seen under normal conditions only after 120 h. The thin layer of epithelium can now perform its function of sealing the tissue. The replacement of the substances lost from the stroma corneae can then proceed under this protective epithelial cover. In the first phase of wound healing therefore the multilayered epithelium behaves in principle as the single layered epithelium (Schuerholter & Honegger 1975, Nelson & Reel 1975, Hirsch et al 1975, Horn et al 1977). The sliding movement to cover the tissue defect appears to be a unique nature of the corneal epithelial and endothelial cells.

In this study (as in the studies using the transmission electron microscope (Kuwabara et al 1976)) the first cell movements were seen after 1 h. What induces the epithelial cells to slide in the described fashion is as yet not clear. The question why sliding cells possess at one time smooth or wave-like cell membranes and at another time send out coral like or pseudopodia like processes cannot be answered with the help of the scanning electron microscope. Microfilaments as well as intracellular pressure are reported to play a role in the sliding movement (Hirsch et al 1973, Dupasquale 1975, Wohlman & Allen 1968, Taylor et al 1976, Gannon & Anderson 1977, Gipson 1977). Intracellular glycogen provides the necessary energy for the cell movement (Hennighausen et al 1972, Thoft & Friend 1977).

In purely epithelial wounds the stability of the association between the epithelial cells is regained after a week at the latest (Pfister 1975, Hark & Zimm 1977, Long 1976). If however the basement membrane is also damaged the adhesion between the epithelial layer and the stroma corneae is achieved later even though the regeneration of the basement membrane probably begins shortly after the formation of the epithelial cells starts. The first evidence of regenerated basement membrane using the transmission electron microscope was found between 3 and 19 days (Khodadoust et al 1968, Blumcke et al 1969, Pulhorn & Thoft 1977, Kenyon et al 1977). The results using the scanning electron microscope show clearly the loose connection between the sliding epithelial cells and the basement membrane and underline the importance of the basement membrane.

In summary the main role of the epithelium in reparative processes of the cornea is the restoration of the integrity of the protective epithelial cover. This is achieved

most rapidly by sliding which enables the superficial cells with appropriate organisation to protect the cells of the stratum germinativum from further damage. The basal cells can then take over the control of the repair of the damage.

### Acknowledgment

This investigation was realized in cooperation with the Institut für Elektronenmikroskopie der Medizinischen Hochschule Hannover. The author is greatly indebted to Prof. Dr. E. Reale for advice.

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# HISTOCOMPATIBILITY AND CORNEAL GRAFT ENDOTHELIUM

BY

PEKKA RUUSUVAARA

Endothelia of 102 clear human corneal grafts in 93 patients were photographed *in vivo* with a specular microscope. The relation of the endothelial cell density to the HLA and ABO compatibility of the donor/recipient pairs were studied. In addition the effects of vascularization and reversible rejection on the endothelial cell density were analysed.

A negative correlation was found between the HLA incompatibility and the endothelial cell density of the graft. The group with 0-1 (2) mismatches had the highest endothelial cell density ( $1356 \pm 210$  cells/mm<sup>2</sup>). This result was better than in the group with 2 (3) mismatches, but the cell difference was not statistically significant. A statistically significant difference in average endothelial cell densities was found between the groups with 0-1 (2) mismatches and with 3-4 mismatches, the latter having  $880 \pm 475$  cells/mm<sup>2</sup> ( $P < 0.01$ ). There was also a statistically significant difference in average endothelial cell densities between the group with 0-1 mismatch and the untyped group ( $P < 0.01$ ). The difference in cell densities between the group with 0 (1) mismatches and the more incompatible groups together (3-4 mismatches and the untyped group) was statistically almost significant ( $P < 0.05$ ). The ABO-compatible pairs had 18% more endothelial cells than the ABO-incompatible pairs. The difference was not statistically significant, but when the ABO-compatible pairs were compared with all the others (incompatible and untyped) the difference in mean cell density between the two groups was statistically almost significant ( $P < 0.05$ ). Comparison of the effect of tissue incompatibility on graft endothelial cell density separately in the patients with vascularized and non vascularized

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corneas showed that in both categories the endothelial cell density was highest in the group with 0-1 (2) mismatches.

In 8 patients with a reversible rejection episode the average cell density was 90% less than in those with an uneventful postoperative period. The importance of rejection on the endothelium in some special cases is discussed.

**Key words:** cornea - corneal graft endothelium - graft rejection - histocompatibility - corneal vascularization - specular microscopy

In efforts to obtain a clear and functioning corneal graft even in difficult vascularized cases, ophthalmic surgeons have chosen the most viable material available from young donors. The immunological causes of graft failure in non vascularized cases have been neglected. Most authors believe that the immunological privilege of the cornea makes matching of donor and recipient tissues of reduced value in corneal grafting (Allansmith et al 1971). On the other hand there is some evidence that in the human cornea the immunological privilege is relative rather than absolute (Jones 1973, Polack 1973, Batchelor 1976, Vannas et al 1978).

Reports of the influence of ABO incompatibility on the results of corneal transplantation are inconclusive. But among the ABO-compatible transplantations clear grafts were significantly more frequent among the more HLA-compatible than among the less HLA-compatible grafts (Ehlers & Kissmeyer-Nielsen 1973).

This series was not large and the number of good matches (i.e. 3-4 shared HLA antigens) was very small. Cibbs et al (1973) observed indications that in grafts transplanted to vascularized corneas greater HLA incompatibility was associated with a higher risk of rejection.

In an earlier paper (Vannas et al 1976) we studied the importance of histocompatibility in corneal grafting using HLA typed donor-recipient pairs. We showed that in grafts with compatible donor material (0-1 mismatch) rejection occurs only exceptionally as compared with incompatible material. We have therefore continued to select tissue typed cryopreserved corneas for transplantation. The purpose of HLA matching is to minimize graft incompatibility. Donor antigens that are shared by recipient will obviously not harm the graft but antigens possessed only by the donor may possibly lead to rejection.

The essential point for the survival of the graft endothelium may possibly be its HLA and ABO compatibility. Destruction of the endothelium is the first of a series of clinical changes in the graft that culminate in its opacification.

Earlier studies with the specular microscope have not shown any significant difference in endothelial cell density between non-rejected and rejected but still partially clear corneal grafts (Bourne & Kaufman 1976b, Sato 1978).

In this study specular photography has been used for the first time to measure

whether there is a correlation between the endothelial cell density of the corneal graft and the degree of histocompatibility between donor and recipient. In addition the effects of corneal vascularization and rejection on endothelial cell density have been analysed.

## Patients and Methods

**Patients.** The series presented comprised 102 penetrating keratoplasties in 70 patients. Of these grafts, 7 were bilateral and 3 were regrafts. The follow-up study was made in 1976-78. The follow-up periods varied from 1 month to 11 years, averaging 3 years. The patients ranged in age from 16 to 77 years and the donors from 8 to 64 years. Among the patients the ratio of men to women was 40:60.

**Operative method.** The operations were performed mostly by the same two surgeons (SV and HH). The graft sizes varied from 7 to 8 mm. As suture material a nylon monofilament was used for continuous or single sutures. In the earlier cases (40) virgin silk sutures were also used.

Postoperatively, as prophylaxis, all patients received corticosteroid drops in small doses perorally. When the cornea was heavily vascularized or signs of rejection could be seen the patient was also treated with immunosuppressants (Imurel®).

**HLA typing and ABO blood groups.** HLA antigens were determined from peripheral blood lymphocytes of the recipient and of those donors who had been bedridden alive. Usually lymph nodes taken at autopsy provided viable lymphocytes for HLA typing of necro-donors. Typing was done routinely by the microcytotoxicity technique (Amos *et al.* 1969) each HLA position being represented by 2-4 parallel antisera of local and/or international origin. Corneal donor recipient pairs designated by 0-1 (2) or 2 (3) mismatches means that there may have been 2 or 3 mismatches owing to the possibility of one undetected antigen. When only 3 HLA antigens are found the probable reason is heterozygosity.

According to the degree of histocompatibility, i.e. the number of mismatches between donor and recipient, the series was divided into four groups. The first group contained 44 grafts with 0-1 (2) HLA mismatches, the second group 11 grafts with 2 (3) mismatches and the third group 11 transplants with 3 or more mismatches. The last group comprised 23 untyped donor eyes.

The ABO blood group was determined in 70 donors of these 44 transplants.

Table I

Endothelial cell densities  $\pm$  s.d. in transplants of different diagnostic groups at follow up

Diagnosis of patients	Number of cases/vascularized corneas	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (year)	Endothelial cell density (cells/mm <sup>2</sup> )
trachoma	60/ 3	(22) 48.5	43.7	1080 $\pm$ 48
other dystrophies	8/ 3	53.0	7.6	994 $\pm$ 393
herpes	7/ 6	45.9	47.1	1119 $\pm$ 199
tuberculosis or syphilis	3/ 1	46.0	66.7	1133 $\pm$ 496
syphilis or leishmaniasis	9/ 7	(8) 47.4	4.8	1338 $\pm$ 595
other inflammations	11/10	50.0	8.1	1554 $\pm$ 500
abrasion trauma				
unknown	4/ 4	58.3	46.3	1344 $\pm$ 600

compatible and 11 incompatible with the recipient and 22 donors were untyped. The series of graftings was divided into three groups according to ABO compatibility.

**Donor material** The donor series has been analysed in an earlier paper (Ruusuvaara 1979) according to corneal preservation, donor age, cadaver time and postoperative period. Three methods of preservation have been used to store the donor corneas. Altogether 41 corneas were used fresh or after moist chamber storage. A total of 42 corneas had been cryopreserved (Capella et al. 1967, 1972) and 19 had been used after intermediate term preservation in Mh medium. The proportion of Mh medium stored corneas in each compatibility group was about 20%. The observation periods were about 5 years for the 3-4 mismatches and untyped pairs and rather less than 2 years for the more compatible pairs.

**Endothelial cell counting** The specular microscope (Seyber Inc.) was used to photograph the central corneal graft endothelium (Laing et al. (1975), Bourne & Kaufman (1976a)). The magnification of the negative was  $\times 100$  and the final magnification of the positive  $\times 500$ . The endothelial cell population was counted from an area corresponding to 0.01 mm<sup>2</sup> on the endothelium. In most cases counts were made from three to five different micrographs and the mean was taken as the result.

**Diagnosis and vascularization of the recipient cornea** Table I shows the numbers of recipients in the different diagnostic groups and the proportions with vascularized corneas. The largest diagnostic group ( $N = 60$ ) consisted of patients with keratoconus: only three corneas in this group were vascularized. The other dystrophies comprised Salzmann's, Croenoev's and Fuchs' dystrophies: of these eight corneas three were vascularized. So these two groups consisted mostly of avascular recipient corneas. The other patients had postinflammatory manifestations. In nearly all the cases of postinflammatory changes and trauma the recipient cornea were vascularized.

In most of the recipients the limbus had been investigated by fluorescein angiography before the operation. Corneas were classed as vascularized if ghost vessels or one or more larger vessels could be seen. According to this division 51 recipient corneas were vascularized and 68 non vascularized (Table I).

**Rejection** Eight patients had had a reversible rejection episode before photography but their corneas were clear enough for specular micrography. Thus it was possible to study the effect of the rejection episode on the endothelial cell density of the graft.

Statistical analyses were made by Student's *t* test to ascertain the effects of various factors on the parameters studied.

## Results

### Recipient diagnosis and graft endothelial cell density

The average endothelial cell density of the 102 transplants was  $1169 \pm 418$  cells/mm<sup>2</sup> with a mean donor age of 49.1 years.

The recipients with keratoconus had a mean endothelial cell density of  $1040 \pm 478$  cells/mm<sup>2</sup> in the graft. The mean endothelial cell densities for recipients with postinflammatory corneal diseases are to be seen in Table I.

### Effect of HLA incompatibility on graft endothelial cell density (Table II)

Analyses of the effect of tissue incompatibility on the graft endothelial cell density indicated that the difference in mean endothelial cell density between the 0-1 (19) and 2 (3) mismatch groups was more than 200 cells/mm<sup>2</sup> but statistically the difference is not significant. Statistical significance in average endothelial cell densities was found between the groups with 0-1 (2) mismatches and with 3-4 mismatches ( $N = 11$ ) the latter having  $880 \pm 475$  cells/mm<sup>2</sup> ( $P < 0.01$ ). There was also a statistically significant difference in average endothelial cell densities between

Table II

Effect of HLA incompatibility on the endothelial cell density of the transplants

HLA incompatibility (mismatches)	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cell/mm <sup>2</sup> )
0-1 (9)	44	(42) 48.3	49.1	1556 ± 510
2 (3)	22	(21) 50.8	40	1146 ± 413
3-4	11	(11) 47.5	38.3	850 ± 475
Untyped	25	(22) 49.7	40.9	989 ± 343
Total	102	(96) 49.1	40.8	1169 ± 478

Table IIIa

Effect of HLA incompatibility on the endothelial cell density of transplant patients with keratoconus and with other diseases

HLA mismatches	Conus or other DG	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cell/mm <sup>2</sup> )
0-1 (19)	conus	24	(23) 48.8	34.3	1913 ± 419
	others	20	(19) 47.6	51.4	1111 ± 414
2 (3)	conus	14	(13) 52.3	31.7	1087 ± 414
	others	8	(8) 48.3	50.8	1948 ± 940
3-4	conus	11	(6) 41.7	33.4	611 ± 311
	others	11	(10) 54.6	43.6	1100 ± 671
Untyped	conus	16	(15) 47.2	33.6	1001 ± 381
	others	9	(9) 53.4	51.9	855 ± 283
Total	conus	60	(57) 48.5	33.5	1040 ± 458
	others	49	(41) 49.9	51.4	1291 ± 484

the group with 0-1 (2) mismatches and the untyped group ( $N = 9$ ,  $P < 0.01$ ). The difference in cell density between the group with 2 (3) mismatches and the more incompatible groups together (3-4 mismatches and the untyped group) ( $N = 36$ ) is statistically almost significant ( $P < 0.05$ ).

Table IIa shows that the importance of histocompatibility is almost as clear among the patients with keratoconus as among those with other diagnoses and with a higher proportion of vascularized corneas. In both groups the same tendency was observed for endothelial cell density to decrease with increasing incompatibility.

#### Effect of vascularization on graft endothelial cell density (Table III)

All corneas with blood vessels in the FAG of the cornea were regarded as vascularized. The vascularized corneal bed group comprised 34 transplants with a mean endothelial cell density of  $1297 \pm 469$  cells/mm<sup>2</sup>. The non vascularized corneal group comprising chiefly corneal dystrophies with a total number of 68 transplants had a mean cell density of  $1106 \pm 474$  cells/mm<sup>2</sup>. The difference in cell density between the vascularized and non vascularized groups was statistically almost significant ( $P < 0.05$ ).

#### The relative importance of HLA incompatibility and vascularization (Table IV)

The patients were divided first according to vascularization into those with non vascularized and vascularized corneas and then into subgroups according to tissue incompatibility. In both vascularized and non vascularized groups the compatible grafts had clearly more endothelial cells than the incompatible or untyped ones. Among the patients with vascularized corneas no difference was found

Table III  
Endothelial cell density of transplants in vascularized and non vascularized recipient corneas

Recipient corneal bed	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells/mm <sup>2</sup> )
Vascularized	34	(34) 0.7	49.9	$1297 \pm 469$
Non vascularized	68	(69) 48.4	37.1	$1106 \pm 474$
Total	102	(96) 41.0	40.9	$1169 \pm 474$

Table II

Effect of HLA incompatibility on the endothelial cell density of the transplants in patients with vascularized and non vascularized corneas

HLA incompatibility (mismatches)	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells/mm <sup>2</sup> )
<i>Vascularized</i>				
0-1 (2)	16	(16) 45.9	48.6	1491 ± 591
2 (3)	7	(7) 51.4	46.1	1229 ± 993
3-4	4	(4) 51.8	46.5	1069 ± 770
Untyped	7	(7) 58.7	46.6	944 ± 178
Total	34	(34) 50.4	48.3	1097 ± 469
<i>Non-vascularized</i>				
0-1 (2)	28	(26) 49.7	38.3	1299 ± 411
2 (3)	15	(14) 50.4	33.1	1109 ± 463
3-4	7	(7) 45.1	39.3	1059 ± 91
Untyped	18	(15) 45.5	37.7	1000 ± 399
Total	68	(60) 48.4	37.1	1108 ± 444

between the groups with 2 and with 3-4 mismatches. However, these groups comprised only 7 and 4 patients, which is too small a number for statistical analysis. In the non-vascularized group, the difference between the groups with 2 and with 3-4 mismatches is statistically almost significant ( $P < 0.05$ ), the cell densities being  $1109 \pm 463$  ( $N = 15$ ) and  $1059 \pm 235$  cells/mm<sup>2</sup> ( $N = 7$ ).

This analysis thus shows that regardless of whether the patient's cornea was or was not vascularized, the average endothelial cell density of the transplant among compatible pairs (means 0-1 (2) mismatches) was clearly above the mean cell density of the whole series. Also, the mean cell densities in the groups with 2 mismatches were close to the mean for the whole series.

HLA compatibility and graft endothelial cell density

Table V shows the cell numbers in different ABO-compatibilities. Although the ABO-compatible group had 18% more endothelial cells than the ABO-incom-

Table V

Effect of ABO blood group compatibility of the endothelial cell densities of the transplants

Blood group compatibility	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells/mm <sup>2</sup> )
(ABO) Compatible	69	(66) 50.0	49.3	1298 ± 40
(ABO) Incompatible	11	(11) 48.5	51.1	1023 ± 36
Untyped donors	22	(19) 46.3	40.0	1011 ± 30

patible group the difference was not statistically significant. When the ABO compatible pairs were compared with all the others, the cell densities of the two groups differed almost significantly ( $P < 0.05$ ).

Effect of rejection episodes on graft endothelial cell density (Table VI)

Eight patients in this series had had a reversible rejection episode, but the cornea had cleared sufficiently for photography of the endothelium. In this small series of rejected transplants the mean endothelial cell density was  $945 \pm 95$  cells/mm<sup>2</sup> and in the grafts with an uneventful postoperative course ( $N = 94$ ) the cell density was  $1188 \pm 188$  cells/mm<sup>2</sup>. Thus although there was 20% fewer endothelial cells/mm<sup>2</sup> in rejected grafts than in normal ones, the difference in cell density was not statistically significant.

Table VI

Effect of reversible rejections on the endothelial cell densities of the transplants

	Number of transplants	Mean age of donors (years) Number of known donors ( )	Mean age of recipients (years)	Endothelial cell density (cells/mm <sup>2</sup> )
Rejection	8	(5) 45.1	53.3	935 ± 94
No rejection	94	(89) 49.4	41.6	1188 ± 188
Total	102	(94) 49.1	40.9	1111 ± 188



observation period after rejection

In one patient with keratoconus the endothelial cell density was clearly affected by rejection (Fig. 1) occurred three years after transplantation and 2 months after the rejection episode the cell density was 1120 cells/mm<sup>2</sup> which was included in the results for the series of 102 patients (Fig. 2a). Two years later the endothelial cell density had decreased to 550 cells/mm<sup>2</sup> (Fig. 2b). The later photograph is just a routine check up and not regarded as adequate for changing of the statistics. In the present study the average follow up period after rejection is 2.5 years.

## Discussion

In Bourne & Kaufman's (1976b) series of untyped donors the mean endothelial cell density correspond with the result of untyped pairs in this study ( $989 \pm 313$  cells/mm<sup>2</sup>) but better cell densities were found in more compatible pairs.

The negative correlation between the incompatibility of donor recipient pair and the endothelial cell density of the graft tells us that the best possible result is obtained with 0-1 mismatch pairs. Endothelial cell loss is considerable in pairs involving 3-4 mismatches or untyped donors. Corneal antigens result in some kind of subclinical rejection episodes which probably occur to some degree in every compatible transplant. According to Khodadoust & Silverman (1969) all corneal grafts may be rejected. But for the maintenance of clarity and deturgescence in the graft the most important layer is probably the endothelium. The less compatible the



FIG. 1

Fluorescein angiography of the cornea. A rejection episode with vascularization in the upper part of the graft. The microphotograph taken for use in the text is a copy.



*Fig 2A*



*Fig 2B*

- A Central corneal endothelium of the same cornea as in Fig 1 seen 9 months after the rejection. The mean cell density is 1120 cells/mm<sup>2</sup>. Two different fields are shown together.
- B The same central corneal endothelium as in Fig 2A seen 2 years after the rejection. The mean cell density is 770 cells/mm<sup>2</sup>.

transplant the greater the endothelial cell loss due to subclinical rejection. Conclusive evidence about the effect of latent rejection on endothelial cell density will probably require long follow up periods.

In our earlier study (Vannas et al 1976) we noticed the clinical importance of HLA typing in corneal grafting. In that series rejection episodes were seen to occur more often in the untyped and the less compatible donor recipient pairs. The present study shows that a correlation exists between endothelial cell loss and degree of HLA incompatibility.

There are of course other factors which may affect the endothelial cell density of the graft e.g. donor age, cadaver time and postoperative period (Ruusuvaara in press). One of the most important is donor age (Bourne & Kaufman 1976b, Sato 1976, Ruusuvaara in press). As Table II shows, between the groups based on the number of mismatches the differences in mean donor age were not statistically significant and cannot account for the difference in endothelial cell density between the different groups. The mean age of the recipients was also almost identical in the different groups. The postoperative periods were longer (about five years) for the more incompatible pairs (3-4 mismatches, untyped) than for the compatible pairs (rather less than two years). Although this will tend to impair the results for the incompatible pairs, it does not explain the whole difference. Still longer follow up for compatible grafts are needed to show how much better the cell survival will be in the long run.

In regard to the relation between the diagnosis of the recipient and the endothelial cell density of the transplant, it was found that the cell density of the graft was lower in the patients with keratoconus than in the other patients. Another point that emerged was the low endothelial cell densities in the patients with other corneal dystrophies. The patients with keratoconus or other dystrophies had fewer cells in their corneal grafts than the other patients, and this poorer result was independent of vascularization. But as Table IIa shows, in the patients with keratoconus a good HLA match seems to be nearly as important as in the patients with other diseases, in whom the cell density of the graft clearly decreased with increasing incompatibility. So it seems that regardless of diagnosis, tissue compatibility is of great value (Chandler & Kaufman 1974).

The importance of ABO compatibility for the clinical results of corneal transplantation is controversial. In previous studies (Meyer 1966, Alberth 1968) the only precaution taken to avoid immune reactions was the determination of ABO compatibility. HLA antigens are present in the human cornea (Ehlers & Ahrens 1971) and so HLA compatibility may also be an important factor in immune reactions against corneal grafts.

In a follow up study of ABO-compatible transplantations Ehlers & Kissmeyer-Nielsen (1973) found that clear grafts were significantly more frequent among the ABO-compatible pairs. But in their series pairs with only 0-1 or 2 mismatches were uncommon, because no attempt had been made to select good matches. In fact their series was randomly selected with respect to histocompatibility matches.

In this series the endothelial cell density in the ABO-compatible donor-recipient pairs was higher than in the small number of ABO incompatible pairs. However, the difference was not statistically significant. But when the ABO-compatible patients are compared with the ABO incompatible and untyped patients, the graft endothelial cell densities show a statistically significant difference.

Vascularization of the recipient cornea is believed on good grounds to jeopardize the success of keratoplasty. Interestingly in a series of 343 keratoplasty patients Cipelli *et al.* (1972) found that vascularization of the cornea seemed to play a relatively small role in the long term clinical prognosis. They showed that even eyes with severe vascularization may do well after keratoplasty and the prognosis for such eyes does not differ greatly from that for eyes without corneal vascularization. The present study shows that the endothelial cells of the graft survive quite well in a vascularized bed. One explanation is that half of the patients with vascularized cornea belonged to the 0-1 (2) mismatches group. Comparison of the effect of tissue incompatibility on the graft endothelial cell density separately in the patients with vascularized and non vascularized corneas (Table IV) showed that in both of these categories the endothelial cell density was highest in the group with only 0-1 (2) mismatches. A compatible cornea thus seems conducive to a good result whether the cornea is vascularized or not.

In spite of anti-inflammatory therapy eight patients had had reversible clinical rejection episodes. The average cell density in these patients at follow up was about 20% less than in the patients without rejection. After reversible rejection some patients had very low cell densities in the central corneal endothelium. In one patient with keratoconus vascularization appeared in the upper part of the transplant 3 years postoperatively and the rejection seemed to have a distinct effect on the central corneal endothelial cell density (Fig. 2a and b).

This indicates that rejection does affect central cell density but the change is visible only after a considerable period, perhaps even years. The type of rejection and the corneal layer affected in the rejection may also be decisive.

Although the difference between cell densities in patients with and without rejection is not statistically significant it seems that rejection episodes may decrease the central endothelial cell density to some degree though in most cases rejection is limited to the peripheral part of the transplant. Bourne *et al.* and Sato had previously reported similar effects of rejection on the endothelium. So it is very important to prevent a rejection episode by anti-inflammatory therapy as early as possible.

In this series it seems that pairs with 0-1 HLA mismatch have the best chances of endothelial survival. The results also suggest that untyped pairs or pairs with 3-4 mismatches suffer from subclinical rejection with a subsequent decrease in endothelial cell density to levels that are clearly lower in the mismatched and untyped groups than in the other groups.

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## HLA TYPES IN CORNEAL DISEASES

BY

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FLEMMING KISSMEYER NIELSEN

Evaluation of the results of HLA typing of 187 patients grouped into herpetic keratitis (37) non herpetic keratitis (43) keratoconus (49) endothelial dystrophy (23) stromal dystrophy (13) lues (5) and injuries (24) failed to show convincing deviations in any of the groups from a normal control series (900 persons). Yet as for the herpes group a rise in B<sub>2</sub> must strongly be suspected. The data are presented and possible implications are discussed.

**Key words:** HLA — keratoconus — keratitis — Fuchs' endothelial dystrophy — stromal dystrophy.

Within medicine interest is increasingly being focused on HLA tissue typing. The outcome of kidney grafting depends on the degree of HLA compatibility between donor and recipient and this applies also for corneal grafting (Ehlers & Kissmeyer Nielsen 1979). Tissue typing has shed light on hereditary, aetiological and prognostic aspects of many diseases. Tissue typing is rather expensive and in order to avoid superfluous repetitions of positive as well as negative studies Siejgaard et al. have taken the praiseworthy initiative of establishing an HLA and Disease Registry. The third report from this registry is to be published in the near future (Ryder et al. in press).

In an earlier paper (Damgaard Jensen & Kissmeyer Nielsen 1978) the results of HLA typing in open angle glaucoma were summarized. No deviations from the normal population were found.

Zimmermann et al. (1977) suggest but do not prove a rise in HLA B<sub>2</sub> in patients with recurrent corneal herpes simplex infection. According to Vannas et al. (1978)

HLA of prognostic significance for the result of corneal grafting in keratoconus is the occurrence of B12 and B27 is significantly higher in the group of graft failure.

Since 1968 we have HLA typed corneal donors and recipients with the purpose of gaining information about the importance of HLA compatibility for the fate of the graft. These results have been published in 1971 and 1973 and now the publication of an analysis of a series of about 200 full-house matched cases is in preparation. The number of cases in the different diagnostic groups are now so great that it allows an analysis of the frequency of the HLA antigens in the diagnostic groups compared with the findings in a large group of normal blood donors.

## Material and Methods

The patients comprising 37 cases of herpetic keratitis, 13 cases of non-herpetic keratitis, 42 cases of keratoconus, 23 cases of Fuchs endothelial dystrophy, 24 cases of ulcers, 13 cases of stromal dystrophy and 5 cases of syphilitic corneal changes were compared to a large series of normal blood donors (2460) slightly fewer for the antigens. The HLA types were determined by the cytotoxicity test according to Karmøer Nielsen & Hærbøe (1967).

The calculations were undertaken by electronic computer and chi-square test with Yates correction was used except where the numbers were too small in which case Fisher's exact test was applied.

## Results

The results appear from Table I which shows the 2P values at and below the 5% level. The rest of the results will be delivered on application to the HLA and Disease Laboratory, address: A. Stejgaard M.D. Tissue Typing Laboratory, BL 1911 Copenhagen, Blegdamsvej 9 DK 2100 Copenhagen O Denmark. It appears that the distribution of 2P values does not deviate from what might be expected according to M. Grumet (multiplication of the 2P value by the number of antigens compared) (= 31) (Grumet et al. 1971) eliminates any trend of significance.

## Discussion

Calculations (31 antigens and 7 diagnostic groups) were made and the results were about the 1% level in only 3 cases (2 in the keratoconus group and 1 in the Fuchs dystrophy group). This was what one might have anticipated, and

Table I

Deviations (compared to a healthy control group) of HLA types of various disease groups of corneal diseases

Diagnosis	HLA type	No of patients	No of positive patients (%)	No of controls	No of positive controls (%)	P
Herpetic keratitis	A2	37	14 (37.8)	986	174 (17.6)	0.003
	B5	37	8 (21.6)	986	119 (12.0)	0.003
	CW3	28	5 (17.8)	986	80 (8.1)	0.001
Non herpetic keratitis	no deviation					
Keratoconus	B7	42	5 (11.9)	986	89 (9.0)	0.114
	B15	40	14 (34.9)	986	74 (7.5)	0.010
Fuchs endothelial dystrophy	B12	23	11 (47.8)	986	74 (7.5)	0.001
Stromal dystrophy	A28	12	4 (33.3)	986	74 (7.5)	0.036
	B7	13	0 (0.0)	986	89 (9.0)	0.036
	B10	12	4 (33.3)	986	74 (7.5)	0.001
Fuchs	no deviation					
Injuries	No deviation					

the primary conclusion is accordingly that the results are entirely negative. However a priori one could have expected the frequency of the antigens on deviating from the normal population in case of keratoconus (hereditary association to other diseases) herpetic keratitis (possibly an immunological defect) Fuchs endothelial dystrophy (defects in the fibrinolytic and the complement system (Bramsen Stenbjerg, 1979)) and possibly in stromal dystrophies (hereditary). These expectations are to some degree fulfilled as the borderline significances were in the keratoconus and the endothelial dystrophy groups. Further investigations are needed to confirm or invalidate the suggestions. As regards herpetic keratitis an elevated frequency of B7 has been reported ( $\chi^2 = 7.13$  2P about 0.01) (Zimmermann et al 1977). Summation of these results with those of the present investigation show a rise in with a chi square of 14.12 corresponding to a 2P value  $< 0.001$ . Provided that Zimmermann et al's findings (1977) is regarded as a preliminary result to be invalidated or confirmed by another similar investigation (such as the present one).



seems justified to regard a rise of B7 in herpetic keratitis as being rather strongly indicated (in that case correction of Crumet is unnecessary and the result of the one-sided P test is  $P = 0.036$ ). This stresses the need to avoid attaching importance to the results of one single investigation, whether they are negative or positive.

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# NON CONTACT SPECULAR MICROSCOPY OF HUMAN CORNEAL ENDOTHELIUM

BY

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The non-contact specular microscope enables a large area of the corneal endothelium to be examined and photographed without any risk of traumatizing the anterior surface of cornea. This non-contact technique is described in detail. Standard deviation of the relative difference between two independent estimates of central endothelial density from 24 eyes was 3.1%. Standard deviation of the relative difference between counts from left and right eyes from 57 subjects was 4.7%. A significant negative correlation between age and endothelial cell density was found ( $r = -0.59$ ,  $P < 0.001$ ,  $n = 76$ ). The interindividual variation in cell densities was found to be larger in the old age group indicating that cell loss during aging varies among different individuals.

*Key words:* specular microscopy — non-contact — cornea — endothelium — age

The technique of visualizing the endothelium by specular illumination with the slit lamp was originally described by Vogt (1920). The magnification available with ordinary biomicroscopes is however not sufficient for a detailed study of the cellular morphology of the endothelium. When Maurice introduced the specular microscope these problems were largely overcome (Maurice 1968) so that it became possible to examine and photograph the human corneal endothelium at high magnification *in vivo* (Liang et al 1975, Bourne & Kaufman 1976, Sturrock & Sherrard 1978). This specular microscopy technique implies applanation of cornea with a dipping cone mounted on the outside of a microscope objective. The applanation is of help in keeping the endothelial cells in focus and allows a high magnification (about  $\times 200$ ).

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This contact technique has several disadvantages however. The applanation requires the use of a topical anaesthetic and the physical contact with the patients eye carries a risk of traumatizing the anterior surface of cornea. This risk is even higher in the case of a diseased cornea where opacity of the cornea may hinder or prolong the examination. Furthermore the area of endothelium observed at a time with the most clinical specular microscopes is rather small making deductions as to the state of the whole cornea difficult.

Only a few attempts have been made to perform high magnification specular microscopy without contact with the cornea. Using a macrophotographing system Brown demonstrated that the endothelium can be photographed using a non contact technique (Brown 1970, Brown & Brown 1974). Photographs obtained with the slit lamp have also been reported (Laule et al. 1978). The endothelial photos obtained were clearly not as good as those obtained by contact specular microscopy ad modum Maurice. Recently a more successful attempt has been reported by Holm (1978). The high magnification in his technique was simply achieved by a horizontally mounted specially fitted microscope supplied with an objective with a long working distance (50 mm). With slight modifications this technique has been adopted by us.

The purpose of the present paper was to evaluate the clinical usefulness of the non-contact specular microscope technique to demonstrate the reproducibility of sampling of endothelial cell densities with this technique and to present data on normal intra and interindividual variations in endothelial cell density. The principles for estimation of endothelial cell density with this technique have been described (Olsen 1979).

## Methods

The non-contact specular microscope (Fig. 1) was made by Preusler Instrument AB, Malmö Sweden after suggestions by Dr Olle Holm, University of Lund, Sweden (Holm 1978). It consists of a microscope tube (A) mounted with a microscope objective (B) with a long working distance. A reflex camera (C) is mounted directly on the tube. The ocular of the camera thus functions as ocular of the microscope. A focusing thread (D) connects the microscope set up to a special bracket (E) designed to fit a Zeiss photo slit lamp.

In the present microscope set up an objective with an extra long working distance (11 mm) was used (Leitz 25 x 0.22). This long working distance permits photographs to be taken without physical contact with the patients eye and furthermore a small angle can be obtained between incident slit illumination and reflected light. The angle used in the present study was fixed at 46° by mechanically fixing the slit lamp and the microscope to each other. This small angle induces a constant error in the horizontal distribution which can be corrected for when endothelial density is estimated (Olsen 1979). The slit illumination is provided by the photo

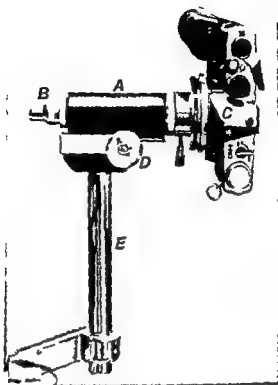


Fig. 1

The non contact specular microscope. A microscope tube B objective with a long working distance C reflex camera D focusing thread E bracket designed to fit a slit lamp.

slit lamp (Zeiss). When photographs are taken both the reflex from the endothelium and the reflex from the anterior surface of the cornea are seen. Due to its large intensity the lower reflex tends to overflow the reflex from the endothelium, an effect which is even more disturbing when the film is exposed. In order to increase the area illuminated on the endothelium relative to the area on the epithelium the ordinary lamp head which gave a narrow beam of light was replaced by a lamp head which gave a more converging slit illumination. A suitable plus lens was inserted between the light source and the plane of fixation and adjusted so that the slit light was focused on the epithelium when photographs of the endothelium were obtained. In this way an increased area on the endothelium can be illuminated.

In order to obtain photographs from a defined and reproducible area on the central cornea a small fixation light was placed between the slit lamp and the microscope (Fig. 1). The long working distance of the objective enables the eye under examination to look at the fixation light which is placed on the angle bisector between incident and reflected light. If the slit lamp, the specular microscope and fixation light are fixed mechanically in each other

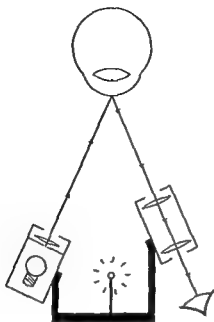


Fig. 2

A fixation light is placed on the angle bisector between incident and reflected light. The angle of reflection on the cornea is defined

the area on the endothelium is defined if the eye is rotated it does not move. If it is moved up-down-back or forwards the reflex is not seen. In case of unpaired vision of the eye under examination but the contralateral eye another fixation light was used to direct the gaze of the contralateral eye. Photomicrographs were taken using a flashing power supply (Siemens) Fast film and white films were used (Agfa Pan 25 24 x 36 mm 13 DIN) with a high magnification (18 x 24 cm) of the negative. All positions were made exactly the same magnification using scale proof plastic paper (Kodak F4 18 x 24 cm). Final magnification was determined by photographing a Burger Turk blood cell counting chamber. A total of about four to eight photographs were taken of each eye. On the basis of the best photographic clarity two photomicrographs were selected on which a total of three 2.5 (1 x 3.5 cm each) were drawn. The area of the frame corresponded to 0.019 mm<sup>2</sup> of cornea. The number of cells within the frames was counted following the counting principle described by Gundersen (Sperling & Gundersen 1981) which allows unbiased estimates to be drawn from small samples. The mean cell count was taken as an estimate of cell density.

The normal subjects included in this study comprised medical students members of the medical staff and patients hospitalized due to squint or senile lens opacities. Eyes with any form of trauma or disease past or present other than squint or senile cataract were excluded.

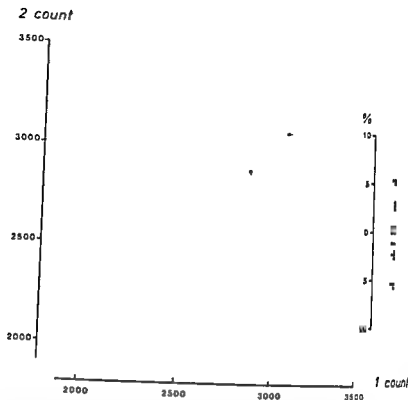


Fig 3

Reproducibility of counting technique demonstrated by comparing two estimates of central endothelial cell density obtained with an interval of 3 to 14 days in 24 normal eyes. Dotted line indicates the graph  $y = x$ . Insert shows the relative difference

$$\left( \frac{2 \text{ count} - 1 \text{ count}}{1 \text{ count}} \times 100\% \right)$$

SD of relative difference is 3.7%

## Results

### Reproducibility of cell counting

Twenty four normal eyes (age range 27-53 years) were photographed and rephotographed after an interval of 3 to 14 days. Endothelial photographs were obtained using the fixation light arrangement depicted in Fig 2. Estimates of endothelial cell density were obtained in a blind fashion by mixing all photographs prior to counting. The result is shown in Fig 3. Endothelial cell density ranged from 2200-3200. The standard deviation of the difference between first and second cell count was 3.7%. To see how effective the fixation light arrangement worked, some photographs were later scrutinized in order to see if it was possible



*Fig. 4*

The same endothelial area rephotographed after one weeks interval. For help of orientation three cells are identified by asterisks. Bar = 100  $\mu$ m

densify the same area. Due to the uniformity of the endothelium this is generally difficult. In one instance however it was actually possible to identify the same cells one week after first examination (Fig. 4). The spatial arrangement of the cells was to be unchanged.

*Correlation with age and difference between right and left eye*

In Fig. 5 the mean cell count of subjects versus age is plotted. When both eyes were photographed a mean of the two counts was employed. A negative relationship appears ( $r = -0.59$ ,  $P < 0.001$ ). A characteristic feature is the large variation seen in 3 age intervals which however is more pronounced in the old age group than in the young age group. If the counts in the age group of 70-80 years is compared to the group of 20-30 years a significant smaller variation is seen in the younger age group (Table I). No differences were observed between cell counts from men or women in any of the age groups. Examples of the variation in cell density in normal retina is shown in Fig. 6.

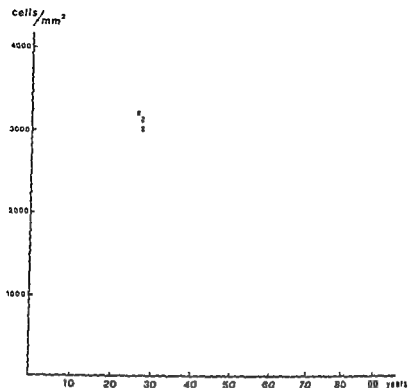


Fig 5

Correlation between age and cell count in 16 subjects ( $r = -0.79$ ,  $P < 0.001$ )

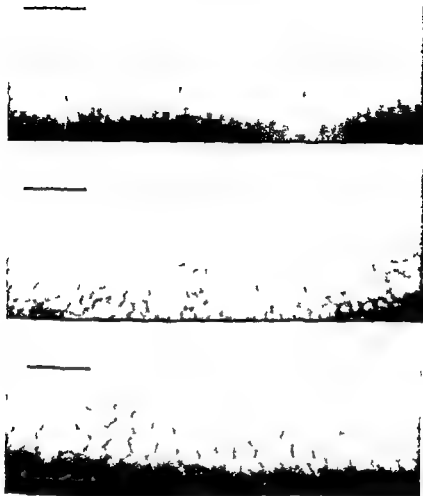
A high concordance between counts from right and left eyes was found (Fig 7), mostly within the counting error (compare Fig 3). Counts from left eyes were found to be slightly (1.8%) lower than counts from right eyes ( $P < 0.01$ ). Standard deviation of the difference between right and left count was 4.7%. The numerical relative difference between right and left eye was found to increase significantly with increasing age ( $r = 0.34$ ,  $P < 0.05$ ).

Table I

Inter individual variation in estimates of endothelial cell density in a young and an old group of normal subjects

Age	n	Cells $\text{mm}^2$		F test
		$\bar{x}$	SD	
20-40	20	3032	248	$F = 9.58$ ( $P < 0.001$ )
70-80	16	2509	398	





*Fig 6*

Normal human corneal endothelium. Top: male 6 years of age endothelial cell density 3060 cells/mm<sup>2</sup>. Center: female 16 years of age endothelial cell density 2960 cells/mm<sup>2</sup>. Bottom: male 70 years of age endothelial cell density 1260 cells/mm<sup>2</sup>. Bar indicates 100 µm.

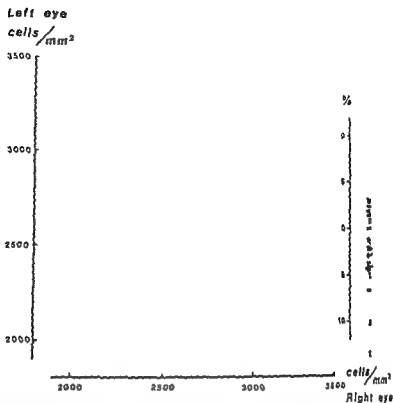


Fig 7

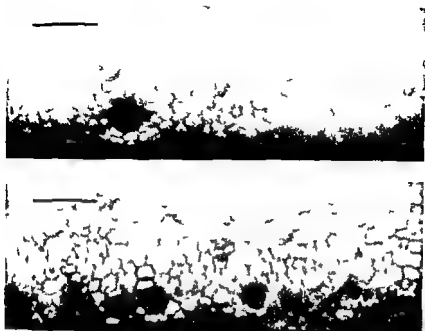
Correlation between estimates of central endothelial cell density from right and left eye in subjects. Dotted line indicate the graph of  $y = x$ . Inset shows the relative difference between the two eyes

$$\left( \frac{\text{left eye count} - \text{right eye count}}{\text{right eye count}} \times 100\% \right)$$

Mean relative difference is  $-1.81\%$  which is significantly different from zero ( $P < 0.01$  basis of the Student distribution). SD of relative difference is  $4.7\%$

### Endothelial morphology

The non contact specular microscope is a sensitive guttae detector of the endothelial cell layer from the plane in the specular reflection. As black areas (Fig. 8). That these black areas really are guttae cell free areas is proved by slightly altering the focusing, whereby the endothelium lining the guttae can be seen. Guttae are often seen in the old age group but isolated minuscule guttae are observed in younger persons (20–30 years of age).



*Fig 9*

Endothelial guttae as seen with the specular microscope. Top: male 63 years old, few guttae visible with the slit lamp. Bottom: female 79 years old, numerous guttae visible with the lamp. Bar = 100  $\mu$ m.

## Discussion

This study has shown the non-contact specular microscope to be a reliable and useful tool in investigating the human endothelium. Due to the lower magnification and the greater angle of observation, it provides a greater view of the endothelium than do most contact specular microscopes (Bourne & Kaufman 1976, Laing et al 1975, Sturrock & Sherrard 1978). This reduces the error when estimates of endothelial cell density are obtained. Furthermore, the non-contact technique enables a highly defined area of the endothelium to be photographed (Fig 2) which increases the accuracy and reproducibility of the method even more.

The decrease in cell density with age has been found by several authors (Laing et al 1976, Bourne & Kaufman 1976, Laule et al 1978, Sturrock & Sherrard 1978), although the magnitude of this decrease differs considerably among different investigators. In a study of necropsy eyes, Irvine & Irvine (1953) found no age-dependent variations in cell density. On the other hand, a very large dependence was reported by Laule et al (1978). The non-contact technique used by the latter

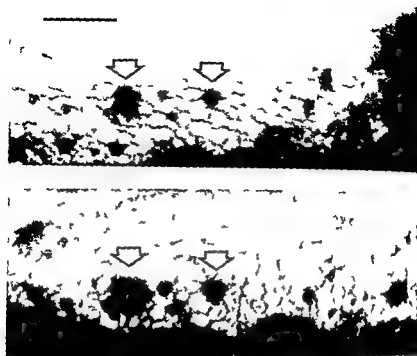


Fig 9

The size of guttae seen in the specular reflex is highly dependent on the angle of observation and the focusing depth which were altered slightly between these two photographs.  
Bar = 100  $\mu$ m

necessitates information regarding the angle of observation in order to correct for distorted dimensions on the photomicrographs (Olsen 1979). This factor was neglected in the study by Laule et al. Sturrock & Sherrard (1978) found an age dependent change in cell density which seems similar to that of the present paper. These authors also demonstrated the reproducibility of their counting technique.

The great variation in cell density among different individuals need to be stressed. The present study also shows this variation to be age-dependent so that the smallest variation is seen in the young age groups. This together with the weak correlation between age and cell density means that the highest cell counts in the old age group are not very different from the counts in the group of 20-30 years. This indicates the cell loss during aging to vary from one individual to another and so actually be rather small in some persons.

Whereas a large variation exists between cell densities from different individuals, the small variation between left and right eye from the same individual is

markable (Fig. 7). Mostly this difference is within the counting error but in some cases a significant difference is found. The age dependence of this difference found in the present study was also found by Laing et al. (1976). The high concordance between left and right eye offers an opportunity of retrospectively assessing endothelial damage following unilateral trauma or disease.

The specular microscope often revealed endothelial guttae which were not seen in the optical section of the ordinary slit lamp. In many instances these guttae were very small, i.e. the area with the absent reflex comprised one or two cells width, but the size was highly dependent on the focusing depth and the angle of observation. Due to the frequent occurrence in otherwise normal corneas, minor guttae were not taken as cause of exclusion from the normal corneas in the present study. Corneas with guttae which were readily seen in the slit lamp were excluded.

In conclusion, it can be said that non-contact specular microscopy is a reliable and producible technique for examination of human endothelium. It is safe and causes no discomfort to the subject due to the non touch technique. Furthermore, combined with a special fixation light arrangement, it enables a defined area on the endothelium to be observed after periods of time. The clinical and experimental usefulness of the non-contact specular microscope is thus fully comparable to, and in some instances even greater than that of ordinary contact specular microscopes.

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# CORNEAL ENDOTHELIAL PERMEABILITY FOLLOWING STORAGE IN MOIST CHAMBER AND MK MEDIUM

BY

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Rabbit corneal endothelial permeability to sucrose and dextran was determined after storage in moist chamber or MK medium. When compared to fresh rabbit corneas there was no statistically significant difference in endothelial permeability to these two solutes after both types of storage for periods up to ten days.

*Acta ophthalmologica* rabbit cornea endothelium · permeability — tissue preservation — tissue storage — corneal preservation

Experimental and clinical work has demonstrated the efficacy of preserving corneas in intermediate term preservative MK medium TC 199 plus 5% dextran (McCarey & Kaufman 1974; Bigar et al. 1975; Stark et al. 1975; Van Horn et al. 1975) however have shown by both trypan blue staining and incubation prior to fixation (scanning and transmission electron microscopy) that a significant number of cat endothelial cells are non viable after storage for periods longer than four days in MK medium. It was the purpose of this investigation to compare the endothelial permeability to non transported non-electrolytes of rabbit corneas stored in MK medium and moist chambers for period up to ten days and thereby assess and compare endothelial barrier function following storage by the two techniques.

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## Materials and Methods

Adult albino rabbits were sacrificed with an overdose of sodium pentobarbital and the eyes were enucleated. Either the entire eye was stored in a moist chamber at 4°C, or a cornea with a scleral rim was removed and stored in MK medium at 4°C. Corneas stored for periods of 4 and ten days by each technique were then atraumatically mounted in a perfusion chamber at 37°C and the endothelial surface and epithelial surface bathed in a modified Krebs-Ringer bicarbonate (KRB) solution (Green & Green 1969) with adenosine (134 mg/l) and reduced glutathione (92 mg/l) (Dickstein & Maurice 1979). In addition the bathing solution contained either sucrose or dextran at concentrations of 10 mM or 1.0 mM respectively. Corneas as prepared above or from freshly enucleated eyes were bathed for ten min in either the KRB, sucrose or KRB dextran solution in order to achieve osmotic equilibrium. The procedure was followed to eliminate the potential influence of bulk osmotic water movement influencing solute movement into the cornea. Mishima & Hedbys (1967) demonstrated that over 90% of thickness change (and hence net fluid movement) was complete within 10 min after exposure to similar osmotic solutions. Thereafter the solution perfusing the endothelial surface was removed and replaced by one containing all of the constituents of the initial bathing solution plus trace amounts of  $^{14}\text{C}$  sucrose (8.1 mCi/mM) or  $^{14}\text{C}$  dextran (1 mCi/mM). Corneas were then bathed for an additional 30 min after addition of the labelled compound to the endothelial perfusing chamber. After 30 min an aliquot from the endothelial perfusing chamber was sampled and the corneas removed. Corneas were washed briefly in three separate aliquots of KRB plus sucrose or dextran, blotted on filter paper and a central 5.5 mm button removed with a trephine. The corneal button was weighed, dissolved in 2 ml Protosol (New England Nuclear Corporation, Boston, Mass.) at 55°C and after cooling and addition of 10 ml of Liquifluor (New England Nuclear Corporation, Boston, Mass.) counted in a Packard Tri-carb liquid scintillation counter. One hundred  $\mu\text{l}$  aliquots from the endothelial perfusion chamber were similarly treated and counted.

The corneal concentration of sucrose or dextran was determined and expressed as  $\mu\text{g}$  solute/mg cornea. Since it was not possible to measure corneal thickness in this system, corneal endothelial permeability was calculated assuming a corneal thickness of 400  $\mu\text{m}$  and a hydration of 70%. The permeability coefficient was calculated using the formula

$$\text{unidirectional flux} = \frac{\text{increase in counts on unlabelled side} \times \text{corneal volume}}{\text{counts on labelled side} \times \text{time} \times \text{area}}$$

Which has the dimensions of cm/sec. Results in corneas stored by the two techniques were compared to those values in freshly enucleated rabbit corneas.

## Results

Sucrose uptake, dextran uptake and endothelial permeability in each group of corneas is shown in Table I. A comparison of moist chamber eyes and MK corneas after storage for four or ten days shows no statistically significant difference in either corneal levels of labelled sucrose or dextran or endothelial permeability to the different solutes when compared to fresh corneas ( $P > 0.05$ ).



Table I  
Cornea endothelial permeability after storage

		Solute uptake ( $\mu\text{g}/\text{mg}$ cornea)	Permeability coefficient $\times 10^{-6}$ sucrose $\times 10^7$ dextran ( $\text{cm}/\text{sec}$ )*
<b>Sucrose</b>			
Fresh		$2.035 \pm 0.279$	$15.69 \pm 1.99^{**}$
Moist chamber	4 d	$1.461 \pm 0.135$	$9.99 \pm 0.90$
	10 d	$1.360 \pm 0.160$	$9.17 \pm 1.08$
VK medium	4 d	$1.779 \pm 0.075$	$12.10 \pm 0.51$
	10 d	$1.760 \pm 0.120$	$11.94 \pm 0.79$
<b>Dextran</b>			
Fresh		$0.235 \pm 0.078^*$	$6.40 \pm 0.72$
Moist chamber	4 d	$0.319 \pm 0.074$	$8.39 \pm 0.90$
	10 d	$0.245 \pm 0.025$	$6.13 \pm 0.68$
VK medium	4 d	$0.251 \pm 0.465$	$6.85 \pm 1.27$
	10 d	$0.240 \pm 0.075$	$6.57 \pm 0.68$

$N = 4$  all experiments

Assuming corneal thickness  $400 \mu$  and hydration  $75\%$

\*  $\pm 1\%$  No significant difference within sucrose group or within dextran group  $P > 0.05$

### Comments

The similar corneal levels of sucrose or dextran at all time periods studied suggests that no alteration of corneal barrier function has occurred in rabbit eyes stored either in moist chambers at  $4^\circ\text{C}$  or in rabbit corneas stored in VK medium at  $4^\circ\text{C}$ . It is known that corneal endothelial permeability to non transported non electrolytes is related to the integrity of the intercellular apical junction rather than to endothelial cell pumping function (Mishima & Trenberth 1968; Kim et al 1971).

It is of interest to note that the endothelial permeability to sucrose and dextran determined in these experiments from corneal uptake of the solutes using assumed corneal hydration and thickness values are close to those values previously reported from determinations made across the endothelium alone which separated two compartments which were subject to sampling (Mishima & Trenberth 1968; Kim et al 1971). The latter procedure offers greater accuracy due to the sampling from the chambers on each side of the membrane. The choice of  $400 \mu$  as corneal thickness is certainly valid for fresh tissue as has been noted on numerous

occasions. MK stored corneas also show normal thickness (which is the basis for the inclusion of dextran in the medium). The moist chamber corneas however are swollen and the value shown in Table I for moist chamber permeabilities increased by a factor of about 25% with this correction there is no statistically significant difference however between these corrected values and those obtained in fresh corneas. Loss of solute across the epithelium is not a factor in these experiments since this membrane is not permeable to these solutes (Green & Green 1973; Mishima & Hedbys 1967).

Previous work has demonstrated that human corneal endothelial permeability to sucrose and urea increased as a function of both time and age of the donor during storage at 4°C in a moist chamber (Hoeftle 1969). It has been demonstrated that the endothelial permeability of older donors increased at a more rapid rate than that of younger donors and that the permeability of a 4 month old donor was unchanged after 80 h of storage (Hoeftle 1969). The unchanged permeability in healthy rabbit corneas used in this investigation may be compared to the unchanged permeability in the 4 month old human cornea. It is currently not known if human corneas stored in MK medium undergo a change in endothelial permeability as do older eyes stored in moist chambers.

This investigation is comparable with previous work comparing moist chamber storage with storage in MK medium (McCarey et al. 1974). That study demonstrated the ultrastructural integrity of the intercellular space and apical junctions of rabbit endothelial cells after storage in either moist chamber or MK medium. Indeed it appears that both ultrastructurally and physiologically the rabbit endothelial cell intercellular junction deteriorates late in the storage process and is unchanged at ten days in rabbit eyes stored in moist chambers or rabbit corneas stored in MK medium. The present data compares with that found for the passive bicarbonate flux (endothelial to stromal surface) across the endothelium of corneas stored as whole eyes in a moist chamber or as isolated corneas in MK medium. In MK solution no change occurred in the measured passive bicarbonate permeability after seven days storage and in moist chamber stored corneas the value increased by about 40%. The passive movement of this ion therefore also indicates that little change occurs during storage (Hull et al. 1979).

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# CORNEAL ENDOTHELIAL CELL DENSITY AFTER AN ATTACK OF ACUTE GLAUCOMA

BY

KIRSI SETÄLÄ

The corneal endothelial cells of 25 patients with unilateral acute glaucoma were photographed with a clinical specular microscope. Photography was performed as soon as (average 6–12 h) the IOP had been lowered with *n*-Acetazolamide and/or Mannitol and topical Pilocarpine therapy and the corneas had become clear. Peripheral iridectomy was performed on the affected eye and prophylactically on the fellow eye in most of these patients. The follow up endothelial photographs were taken 6–24 months postoperatively.

High intraocular pressure lasting 3 days or more lowered the central endothelial cell density. But a rise in pressure lasting from only a few hours to 2 days did not affect the endothelial cell count. Operative glaucoma procedures caused a loss of central endothelial cells of approximately 4.8% in the series. There was a clear correlation between the duration of elevated pressure and the number of central corneal endothelial cells lost.

**Key words:** acute glaucoma – corneal endothelial cell density – corneal endothelial cell loss – specular microscope

High intraocular pressure may lead to dysfunction of the corneal endothelium, which permits excess fluid to enter the corneal layers from the anterior chamber (1). It is not clear whether this is due to the high intraocular pressure itself or to secondary damage to endothelial cell function (Stocker 1971).

The endothelial cells of the patient can be examined and photographed with a clinical specular microscope (Maurice 1966, Laing et al 1975, Bourne & Kaufman 1976). In our previous studies with a specular microscope we observed that high pressure led to a reduction in human central corneal endothelial cell density (1).

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unilateral capsular glaucoma the raised intraocular pressure affected the central endothelial cell count (Vannas et al 1977). In patients with unilateral Posner-Schlossman syndrome the loss of endothelial cells seemed to be due mainly to the recurrent attacks of raised pressure (Setälä & Vannas 1978).

The purpose of this clinical study was to examine the effect of raised IOP attacks on the corneal endothelial cell density in eyes with acute glaucoma. We examined the relation of the endothelial cell density to the duration of the high pressure attacks and the pressure level. The effect of surgical trauma on endothelial cell density was also studied.

Table I

Characteristics of the patients with an acute attack of glaucoma

Case No	Age (years)	Sex	IOP on admission (mmHg)	Duration of attack	Endothelial cell densities		
					affected eye (cells mm <sup>-2</sup> )	fellow eye (cells mm <sup>-2</sup> )	difference (%)
1	64	f	64	3 days	90	133	63
2	78	f	62	14 days	829	9103	69
3	80	f	66	10 days	766	1869	59
4	57	f	62	7 days	1430	9519	41
5	72	f	74	7 days	9484	2699	14
6	80	m	44	2 days	1792	218	9
7	74	m	58	1 day	9103	9241	5
8	49	m	74	13 days	915	911	5
9	61	f	60	1 day	9401	218	4
10	50	f	52	2 days	9760	9470	4
11	69	f	58	1 day	189	1904	4
12	66	m	48	9 days	9999	9905	3
13	58	f	68	7 hours	9622	960	3
14	70	f	64	13 days	9614	2656	9
15	64	f	51	1 day	9379	2407	1
16	70	m	80	12 hours	9991	9918	1
17	60	f	78	12 hours	905	9199	1
18	65	m	76	9 days	2567	267	0
19	65	f	55	1 day	9650	960	0
20	80	f	80	1 day	9159	919	0
21	66	f	70	7 hours	9153	9153	0
22	4	f	80	10 hours	9153	2153	0
23	57	f	55	20 hours	989	260	-4
24	71	f	80	1 day	9241	2283	-9
25	65	f	68	13 days	9650	2567	-3
Mean	66.3		65.3	2.5 days	9161.4	2399.3	9.7

## Material and Methods

The series consisted of 25 patients from Helsinki University Eye Clinic (Table 1): 19 females and 6 males. All but one of the patients had the first acute attack in the affected eye. This patient (No. 8) had suffered from an attack of acute glaucoma of two days duration in the same eye 20 years earlier. The mean age of the patients was 66.3 years. Seventeen patients had the glaucoma attack in the right eye and eight patients in the left eye.

The ocular pain, blurring of vision, vomiting and nausea had lasted three days or longer in five of these patients. The history was shorter for the others. A careful eye examination was performed on all patients; the IOP was measured with a Goldmann applanation tonometer.

All the fellow eyes had normal ocular pressure values and no symptoms with no blurring of vision or ocular pain. These eyes were therefore used as controls.

Owing to the increased intraocular pressure, the corneas were oedematous when the patients were admitted. Visualization of the endothelial cells is not possible when the pressure is high and the cornea hazy. The endothelial photographs were taken when the intraocular pressure had been lowered with intravenous (Acetazolamide or Mannitol) or local (Pilocarpine) therapy.

Peripheral iridectomy was performed within 4–6 days of the pressure attack. prophylactic surgery was also performed on the normotensive fellow eyes where the angle was narrow. Trabeculectomy was performed in the affected eye in only two of the patients. If the values for the coefficient of the facility of outflow ( $C$ ) were under 0.1, a filtering operation was regarded as necessary. The endothelial cells were also photographed 6–24 months postoperatively when possible.

The series included one patient (No. 22) who refused surgery and received no therapy after the acute attack. In spite of this, the pressure remained normal in both eyes. The endothelial photographs were taken two years after the acute attack.

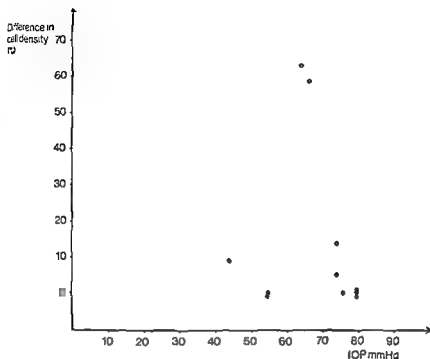
**Procedure for photography of the endothelial cells with a specular microscope**

Several pictures, six on average, were taken of the central cornea in both eyes of each patient. The endothelial cells were analyzed and counted as described in a previous article (Vannas et al. 1977).

## Results

The average endothelial cell count in the affected eye was  $2161 \pm 633$  and in the fellow eye  $2392 \pm 346$  SD. The mean difference was 9.7% ( $P > 0.05$ ) (Table 1).

The intraocular pressure in the affected eye when first measured in the hospital



*Fig 1*  
Effect of the level of IOP on the difference in endothelial cell density between the affected and control eyes

(Table I) ranged from 44 mmHg to 80 mmHg mean  $60 \pm 3$  mmHg. There was no correlation between the level of IOP and the difference in endothelial cell density (Fig 1).

#### Effect of duration of glaucoma attack on endothelial cell count

The duration of the attack varied from 7 h to 14 days. In five patients the glaucoma attack lasted between 3 days and 2 weeks. The endothelial cell density of these five patients was clearly lower in the affected eye than in the normotensive fellow eye (Table I). When the pressure attack lasted less than three days the endothelial cell count was only slightly lower than or about the same as in the control eye. Thus there seems to be a clear correlation between the endothelial cell loss and the duration of the glaucoma attack (Fig 2).

The difference in endothelial cell count between the affected and control eye was greatest in a 64-year-old woman (patient No. 1, Table I) whose glaucoma attack lasted three days. The endothelial cell density in the acutely affected eye was 40 mm<sup>2</sup>; the difference is 63% (Fig 3).

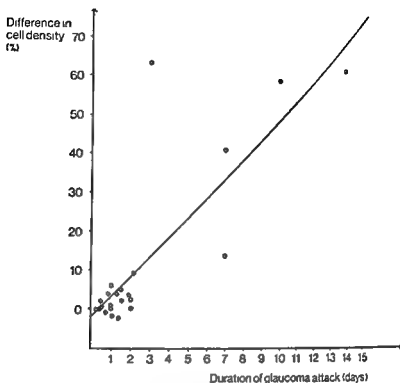


Fig. 2

Effect of the duration of the glaucoma attack on the difference in endothelial cell density between the affected and control eyes

No patient displayed any cupping of the disc or visual field defects during the follow up

In some patients the visual acuity was affected by cataract or macular degeneration. Endothelial cell loss did not correlate to visual acuity.

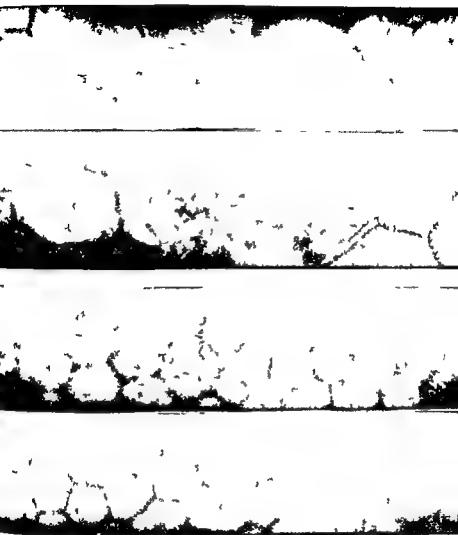
#### Effect of operative procedure on endothelial cell density

The endothelial cell loss after iridectomy was 1.9% in the affected eye and 4.6% in the fellow eye (Table II).

Trabeculectomy was performed on the affected eye in two patients and the mean difference between the pre- and postoperative values in their affected eye was 5%. In the unoperated fellow eye the cell loss seemed to be 0.5% during the observation period (Table III).

The cell loss caused by glaucoma surgery seems to occur during or soon after the operation. No distinct decrease in endothelial cell densities was noted during the 6–24 month postoperative period.





*Fig 3*

61-year-old woman with 3 days raised IOP (patient No. 1). Above the affected eye with 580 cells/mm<sup>2</sup> below the control eye with 1579 cells/mm<sup>2</sup>

**Table II**  
**Effect of operations on endothelial cell density**  
**Endothelial cell density after iridectomy on both eyes**

Case No	Affected eye			Fellow eye			Time from operation (days)
	Before oper	After oper	Diff (%)	Before oper	After oper	Diff (%)	
1	580	538	7	1573	1573	0	15
3	766	621	19	1863	1892	2	8
4	1490	1490	0	2519	2318	8	14
5	2484	2318	7	2898	2650	9	15
8	2015	1964	3	2117	2040	4	
9	2407	2407	0	2518	2518	0	13
11	1822	1822	0	1904	1904	0	15
12*	2822	2490	12	2905	2905	0	1
13	2622	2277	13	2670	2346	12	6
14	2614	2443	7	2556	2573	3	10
18	2567	2449	5	2567	2360	8	
19	2650	2614	1	2650	2484	6	1
20	2152	2070	4	2152	1997	8	1
21	2153	2153	0	2153	153	0	22
23	2788	2545	9	2760	267	7	15
24	2241	2075	7	2243	2213	3	6
25	2650	2573	3	2567	267	0	22
mean	2125	2022	4.9	2567.9	2566.6	4.6	13

\* iridectomy only on affected eye

## Discussion

A rise in pressure lasting at least three days caused a clear decrease in the endothelial cell density of the eye affected by the acute glaucoma attack as compared with the normotensive fellow eye. In eyes in which the duration of raised pressure was shorter from a few hours to two days the differences in endothelial cell density between the affected and control eyes were quite small.

Because of the small number of patients statistical analysis is not possible. The exact duration of raised IOP which causes cell death is perhaps quite variable in different patients. It may vary depending on the age of the endothelium which is affected by the pressure and also on the primary cell density before IOP attacks.

Glaucoma surgery peripheral iridectomy and iridectomy have been suggested to cause lowering of central endothelial cell counts. In this series the mean

*Table III*  
Endothelial cell density after trabeculectomy on affected eye

Case No	Affected eye			Fello eye			Time from operation (months)
	Before oper	After oper	Diff (°)	Before oper	After oper	Diff (°)	
6	1999	1909	4	9185	9111	3	14
15	2319	2941	6	2107	9460	-9	19

No operation

Case No	Affected eye	Fellow eye	After 74 months		Diff (°)	
			Affected eye	Fellow eye		
92	2153	2153	9173	9153	0	0.0

loss of endothelial cells after iridectomy was 4.8% and after trabeculectomy about the same. Any procedure involving surgical entry into the eye seems to cause damage to the endothelial cells.

Today patients with a history of high IOP lasting more than a few days are rare. In hospital the pressure can be lowered within hours with drugs (Pilocarpine iv, Acetazolamide iv, Mannitol).

Endothelial photography was performed soon after admission. Two possibilities were envisaged. The first was that the cells damaged by the acute glaucoma attack would not yet have disappeared and would still appear morphologically normal in the micrograph. The other possibility was that the damaged cells would have died and been resorbed soon after the damage caused by the increased intraocular pressure but that the cornea would not become clear until the remaining living endothelial cells had spread and refilled the gaps left by the dead cells.

Follow up photography was done 6-24 months postoperatively. The control values were still valid because iridectomy had been performed on both eyes (except in one case) for bilateral narrow angles. This follow up photography showed that the endothelial cell loss caused by increased intraocular pressure occurs during the glaucoma attack or at least before the cornea becomes clear. No distinct cell loss caused by the glaucoma attack was seen during the subsequent follow up period of 6 to 74 months.

Recent studies have investigated the extent to which photography of the central corner is representative of the entire cornea. In Blackwell's study (1974) specular micrographs were taken in four areas of the cornea and the central area provided a measurable indication of the general endothelial cell population in normal young adults, normal older adults and post-cataract adults. Only in the artificial intraocular lens group was there a statistically significant difference in cell numbers in different areas.

In acute glaucoma, however, we may assume that the detrimental effect of the IOP affects the whole cornea uniformly and that the situation is quite different from that obtaining after a cataract operation. Therefore, after an acute glaucoma attack and also after iridectomy, the central endothelial cell density should well represent the endothelial cell number.

There was one patient (patient No. 8) in our series whose case history included an acute attack of glaucoma in the same eye 10 years earlier. However, the difference between the cell densities of the two eyes was small. Usually the endothelial cells are quite resistant to high and long lasting IOP attacks (Patient No. 5).

The greatest difference in cell density between the affected and control eyes was found in a 64 year old woman after a glaucoma attack lasting three days. It is noteworthy, however, that the endothelial cell count in the control eye was the lowest in the series. It may be assumed that this patient had some additional disorder of the endothelium and that diseased endothelial cells are more vulnerable to the traumatic effect of intraocular pressure.

It seems from this study that high IOP can lower the endothelial cell density as little as three days. A pressure elevation lasting only from a few hours to two days does not affect the endothelial cell count. For example, the 74 year-old woman with a 10 hour attack (patient No. 22) who refused surgery had no endothelial cell loss after two years follow-up.

These results are in accord with our previous studies on patients with Pinner-Schlossman syndrome. Patients with numerous and long lasting attacks of the disease had a lower endothelial cell density in the affected eye than in its fellow (Setälä & Vannas 1977).

Glaucoma procedures caused a reduction in central endothelial cell density of approximately 5% in our series.

We conclude that the duration of elevated IOP is a more important factor in damage to the endothelium than the height of the pressure.

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# VARIATIONS IN ENDOTHELIAL MORPHOLOGY OF NORMAL CORNEAS AND AFTER CATARACT EXTRACTION

*A specular microscopic study*

BY

THOMAS OLSEN

The central corneal endothelium of 22 normal subjects and 11 unilateral aphakic subjects was photographed with the non-contact specular microscope. The aphakic patients had undergone intracapsular cataract extraction one to four years previously. Endothelial cell densities were estimated. All cells which had been counted were then grouped according to their number of neighbours. The percentages of cells having 4, 5, 6, 7 or 8 neighbours were means  $\pm$  SD)  $0.4 \pm 0.8$ ,  $18.0 \pm 3.4$ ,  $64.1 \pm 6.5$ ,  $15.9 \pm 2.6$  and  $0.9 \pm 0.8$  per cent, respectively in the normal eyes and  $0.8 \pm 1.3$ ,  $20.9 \pm 3.7$ ,  $57.4 \pm 3.6$ ,  $19.6 \pm 3.9$  and  $1.4 \pm 1.2$  per cent in the aphakic group, respectively. The frequency of cells having 5, 6 or 7 neighbours were significantly different in the two groups. Cells with 9 or 10 neighbours were not seen in the normal group but occurred in 2 and 1 of the aphakic eyes, respectively. In the normal group the frequency of hexagonal cells was found to correlate to the cell density ( $r = 0.40$ ,  $P < 0.01$ ) and age ( $r = -0.40$ ,  $2P < 0.05$ ).

*Keywords:* cornea - endothelium - morphology - specular microscopy

The endothelium of the normal cornea comprises a coherent monolayer of large hexagonal cells, the integrity of which is essential for a normal hydration of the cornea. Following various forms of mechanical injury, the endothelium has been stated to recover and restore a normal cellular pattern from one week to 10 months in the rabbit (Chi et al. 1960; Khodadoust & Green 1976; Olson et al. 1977; van Horn et al. 1977). Longer time periods have been observed for the

endothelium presumably more comparable to man in its limited proliferative power (van Horn et al 1977)

If human endothelial cells are damaged immediate healing occurs by spreading and sliding of undamaged cells to cover the defect. Since the proliferative power of the human endothelium is very limited (Kaufman et al 1966) the result is a decrease in the cell density. Among the factors which cause a decrease in cell density is age (Laing et al 1976 Bourne & Kaufman 1976 Sturrock & Sherrard 1978 Olsen 1979) and cataract extraction (Bourne & Kaufman 1976 Hirst et al 1977 Chen et al 1977 Forstot et al 1977)

This study was undertaken in order to investigate whether cell loss leaves permanent changes in the cellular mosaic of the human endothelium. A knowledge of such morphological alterations may be of help in other clinical situations when cell loss is suspected but the cell density is not obviously lowered.

## Subjects and Methods

The central corneal endothelium of one eye of 22 normal subjects and both eyes of 11 unilateral aphakic patients were photographed with the non-contact specular microscope (Olsen 1979). The normal subjects comprised medical students, members of the medical staff or patients with senile lens opacities. The age range was 19–83 years. The aphakic patients had intracapsular cataract extraction one to four years previously. They were photographed successively as they came to have surgery performed on the other side. Age range 38–89 years. None of the normal or the aphakic patients showed any signs of present disease in the cornea.

Endothelial cell densities were estimated as described earlier (Olsen 1979). All cells which had been counted were then grouped according to their number of neighbours. The relative distribution of cells according to their number of neighbours was calculated for each cornea.

## Results

For the total group of normal eyes estimated endothelial cell densities ranged from  $1.10$  to  $3.57 \times 10^5$  cells/mm<sup>2</sup>. A negative correlation was found to the age of the subject ( $r = -0.31$ ,  $0.1 > 2P > 0.05$ ,  $n = 33$ ). Mean estimated cell densities from the aphakic patients appear from Table 1. The difference in cell counts between unoperated and operated eyes ranged from  $-8.7$  to  $-67.1\%$ .

The percentages of cells having 4, 5, 6, 7 or 8 neighbours were (means  $\pm$  SD)  $34 \pm 0.8$ ,  $18.0 \pm 3.4$ ,  $64.1 \pm 6.0$ ,  $1.5 \pm 2.6$  and  $0.9 \pm 0.8$  per cent respectively in the normal eyes and  $0.8 \pm 1.3$ ,  $20.9 \pm 3.7$ ,  $57.4 \pm 5.6$ ,  $19.6 \pm 3.9$  and  $1.4 \pm 1.2$  per cent in the aphakic group respectively (Fig. 1). The aphakic eyes had significantly

Table I  
Endothelial cell density of 11 unilateral aphakic patients  
(means  $\pm$  SD)

Normal eye (cells/mm <sup>2</sup> )	Aphakic eye (cells/mm <sup>2</sup> )	Difference ( )
2736 $\pm$ 399	1749 $\pm$ 697	-36.8 $\pm$ 16.3

higher frequency of cells with 5 and 7 neighbours and lower frequency of cells with 6 neighbours. Cells with 9 or 10 neighbours were not seen in the normal group but occurred in 2 and 1 of the aphakic eyes respectively.

A significant correlation was found between the number of hexagonal cells and the cell density in the group of normal eyes (Fig. 2) ( $r = -0.40$ ,  $0.01 > 2P > 0.001$ ). A weaker correlation was found between the number of hexagonal cells and age ( $r = -0.40$ ,  $0.05 > 2P > 0.01$ ) (Fig. 3).

In the aphakic group the number of hexagonal cells could not be correlated to either time after the operation, endothelial cell density or difference between counts from operated and unoperated eye.

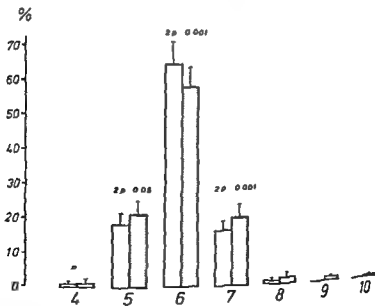


Fig. 1  
Relative frequency of endothelial cells having 4 to 10 neighbours in 55 normal eyes (solid columns) and 11 cataract extracted eyes (cross-hatched columns). Vertical bars represent standard deviation.  
Statistics: unpaired *t* test.



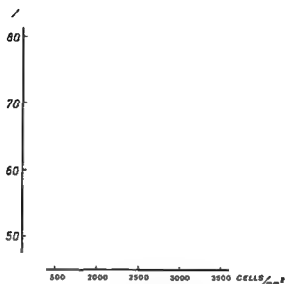


Fig 2

Correlation between the relative number of hexagonal cells and the cell density in 33 normal eyes ( $r = 0.46$ ,  $0.01 > P > 0.001$ )

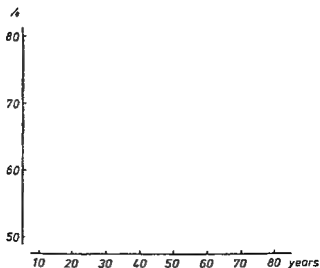


Fig 3

Correlation between the relative number of hexagonal cells and age ( $r = -0.40$ ,  $0.05 > 2P > 0.01$ ,  $n = 33$ )

## Discussion

The present study has shown that a normal cellular pattern is not reformed in 1 to 4 years after injury to the human endothelium. Normally a high correlation exists between endothelial cell counts from left and right eyes (Olsen 1979). The cell loss which occurred at time of cataract extraction is therefore indicated in the difference in counts between the operated and unoperated fellow eyes (Table 1).

The finding of a decrease in the number of hexagonal cells with decreasing cell density indicates that the gradual cell loss during aging also leaves permanent changes in the original largely hexagonal cell pattern. Since age was found to be a minor determinant for the variation in the number of hexagonal cells, this result adds further evidence to a hypothesis that cell loss varies from one individual to another during aging. In other words, the large interindividual variation in cell density seen in old age (Olsen 1979) is an acquired variation and not only a variation present at birth.

The reason why the hexagon is the dominant shape of endothelial cells is unknown. From a geometrical point of view the hexagon is one of the three regular tessellations which can be repeated to cover the whole plane (see Cover 1973) but a triangle and a square could do this as well. It can only be speculated that the characteristics of the hexagon, i.e. its almost circular shape which keeps the total perimeter at a minimum and with neighbouring faces meeting only three at a time, is favorable to cells which must function as a barrier against the stromal imbibition of water.

## Acknowledgments

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## CORNEAL ENDOTHELIAL CELLS IN ESSENTIAL IRIS ATROPHY

*A specular microscopic study*

BY

KIRSI SETALÄ and ANTTI VANNAS

The specular microscope was used to study the corneal endothelium in three patients with essential iris atrophy and three patients with anterior mesodermal disorders with uncertain diagnosis. Pleomorphic and enlarged endothelial cells were found to be typical for essential iris atrophy. The changes were also present in one eye with normal pressure.

In the patients with other types of iridal disorder the endothelial cells were normal in form. In one of these patients high intraocular pressure and surgery had seemingly led to enlargement of the endothelial cells.

According to our findings the specular microscope is a useful aid in differentiating essential iris atrophy from other disorders of the iris.

*Key words:* essential iris atrophy — corneal endothelial cells — specular microscopy — Chandler's syndrome — iridoschisis

Essential atrophy of the iris (EIA) is a rare disease of unknown aetiology characterized by slowly progressive atrophic changes in the iris trabecular meshwork and corneal endothelium and is often accompanied by glaucoma due to changes in the chamber angle (Duke Elder 1966).

Zentmeyer (1918) suggested that vascular sclerosis may be the cause of essential iris atrophy. Heath (1953) expanded this theory with histopathological studies and demonstrated that in far advanced cases the mechanism for the atrophy lies in multiple anaemic infarctions that follow segmental vascular occlusions. However, A.

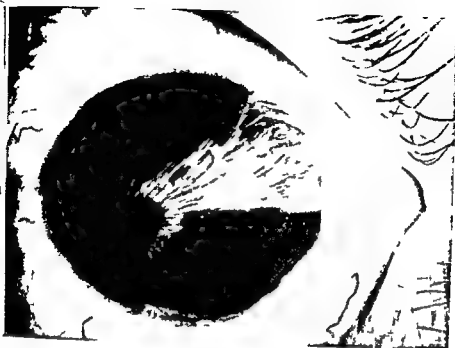


Fig 1 (case 1)

Left eye of the 35 year-old woman with essential iris atrophy of 6 years duration



Fig 1A

Fig 1p Hazy abnormal endothelial cells of the affected left eye of the 35 year-old woman with essential iris atrophy ( $\times 440$ )  
 3.6  $\mu\text{m}$  Contralateral healthy eye with endothelial cells of normal appearance ( $9800 \pm 5 \text{ mm}^2$ ) (Contact specular micrograph  $\times 440$ )



Fig 2 (case 2)

Right eye of the 42 year-old woman with essential iris atrophy. Duration 23 years.

Vannas (1969) using iris angiography to examine eyes with essential iris atrophy found no specific pathological vascular changes.

Recently the hypothesis has been proposed that in essential iris atrophy important roles are played by corneal endothelial degeneration and the growth and contraction of an ectopic endothelial membrane (Vanoff & Fine 1973; Scheer et al 1976; Rodrigues et al 1978; Campbell et al (1978) have proposed a new designation for the syndrome: primary proliferative endothelial degeneration.

In view of the lack of information on the state of the corneal endothelial cells *in vivo* in this syndrome we present data on patients with typical essential iris atrophy. Also three patients with suspicious EIA are presented. The specular photographs excluded the diagnosis.

### Patients and Methods

Our series is presented in Table I. In addition to the regular ophthalmological examination the corneal endothelium was photographed with a contact specular microscope (Bourne & Kaufman 1976) and a non-contact specular microscope by

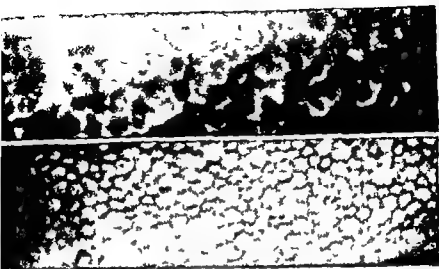


Fig 24 (case 2)

Top: The endothelial cells of the diseased right eye. The outlines of the cells are poorly seen ( $\times 130$ )

Bottom: Endothelial cells of the contralateral healthy eye ( $3000 \text{ cells/mm}^2$ ) (non contact specular micrograph  $\times 130$ )

by one of us (A V). With the non-contact method the primary magnification on the film is  $\times 34$  allowing inspection of a larger endothelial area up to 500 cells in a single picture.

## Results

A 13-year-old woman (Fig 1 Table 1 case 1) noticed an extra pupil in her left eye at the age of 9 and the diagnosis of essential iris atrophy was made a year later. The family and medical histories were negative there being only a history of refractive error. General physical examination revealed no abnormalities. Follow up examination in 1978 revealed normal vision in the right eye. The right eye was normal. With the best correction the vision in the left eye was 0.5. Biomicroscopy showed an endothelium with a fine beaten silver appearance. The anterior chamber was normal in depth. The pupil was markedly distorted. The iris was thin with stretched holes, one strand lying across the middle of the cornea. Gonioscopy revealed broad anterior synechiae. During follow up the eye has remained normotensive. Endothelial photographs were taken of both eyes. Fig 1A shows the endothelial cells of the affected left eye with hazy irregular pleomorphic cells. The contralateral healthy eye of the same patient showed a normal regular mosaic like cell pattern.

The second patient, a 49-year-old woman, noticed at the age of 17 that the pupil of her left eye was distorted towards the 2 o'clock direction (Fig 2). Essential iris atrophy of the left eye was diagnosed in 1909. At follow up examination in 1978 the ocular tension was normal but the iris atrophy showed slow progression. In April 1979 examination showed

normal corrected visual acuities in both eyes. The right pupil was distorted and there was a hole in the iris at the 10–11 o'clock position. Gonioscopy revealed relative angle closure and large synechiae. An elevated IOP of 40 mmHg was measured but was normalized with medication. The optic nerve head was normal. The left eye was normal. In macrophotography the corneal endothelial cells in the right eye were poorly seen. The cells seemed to be hazy and their images hazy. The endothelial cells of the left eye were normal (Fig 2).

The third patient, a 63-year-old woman, noticed at the age of 56 that the pupil of her left eye was displaced (Table I, case 3). The diagnosis of essential iris atrophy was made at the age of 57 (1972) when there were two holes in the left iris and elevated IOP (44 mmHg) in the left eye. The C value was 0.07 ( $\mu\text{l}/\text{min}/\text{mmHg}$ ) and therapy for glaucoma was started (pilocarpine and adrenaline). Follow-up examination in 1978 revealed normal vision in the right eye. The IOP was 16 mmHg and biomicroscopy revealed no abnormalities. The vision of the left eye was 0.25 owing to macular degeneration. There were three holes in the iris. Gonioscopy showed broad adhesions in the areas corresponding to the holes and elsewhere the angle was open. The IOP of the left eye was 30 mmHg. Endothelial photography, iris taken of both eyes. The endothelial cells in the affected eye appeared somewhat hazy and the cell density could not be counted. In the healthy fellow eye the endothelial cells were regular, their density being 2900 cells/ $\text{mm}^2$ .

The fourth case is a 60-year-old woman (Fig 3). From the age of 41 she sometimes noticed dimness in her left eye in the evenings. At that time her left iris became lighter in color. Three years later secondary glaucoma was diagnosed in the left eye. Visual acuity in both eyes was 1.0 with glasses. Biomicroscopy showed some coalescent guttae in the left cornea. The

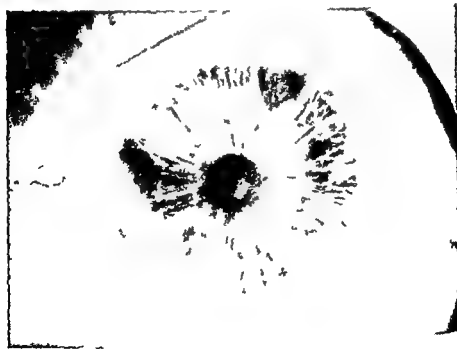


Fig 3

Left eye of the 60-year-old woman with iridochisis of 20 years duration.





Fig 3A

Top: Large endothelial cells in eye with iridoschisis ( $600 \text{ cells mm}^{-2}$ ). The lowered cell density is probably caused by high IOP and intraocular surgery ( $\times 440$ ).

Bottom: The endothelial cells of the healthy contralateral eye of the same patient ( $3000 \text{ cells mm}^{-2}$ ) ( $\times 440$ ).

Anterior chamber was shallow. The colour of the left iris was lighter and there was stromal atrophy. Gonioscopy showed angle closure with broad irregular iridal adhesions. The right anterior segment was normal. The visual fields were free from any scotomas. Ophthalmoscopy showed neither abnormalities nor disc asymmetries. The intraocular pressures were 45 mmHg in the left eye and 16 mmHg in the right eye. Medication with pilocarpine and adrenaline was initiated. In November 1974 (at the age of 56) trabeculectomy was performed on the left eye. Since then the intraocular pressure has been normal without medication. The ocular findings in this patient simulate those of iridoschisis. Iridoschisis usually begins in the 5th decade. It is usually bilateral but may be unilateral. There is localized cleavage of the mesodermal iris stroma into two layers: the anterior leaf separating and disintegrating into fibrils, the distal ends of which float freely in the anterior chamber (Duke-Elder 1966). Endothelial photographs of both eyes were taken in January 1979 and in the right eye the endothelial cells were normal ( $3000 \text{ mm}^{-2}$ ). In the left eye the endothelial cells were very large but quite regular and sharply visualized ( $600 \text{ cells mm}^{-2}$ ) (Fig 3A).

At the age of 19 the fifth patient noticed that her left pupil had become oval (Fig 4). In photographs taken at the age of 15 both pupils were round and symmetrical. The family history was non-contributory and she denied any ocular trauma. At the age of 22 she was examined at Helsinki University Eye Clinic. Visual acuity in both eyes was 1.0 and gonioscopy revealed that the angle was closed at the 8–9 o'clock position, with broad adhesions in the left eye. In April 1979 at the age of 34 the visual acuity in both eyes was 1.0, the ocular pressures were 15 mmHg o.a. and the left pupil was oval as in the photos taken 12 years earlier. The gonioscopy finding was also unchanged. The pupil of the right eye was normal. The micrographs show that the endothelial cells in the two eyes are similar (Fig 4A).

Our last patient is a 38-year-old woman whose left pupil had been oval for as long as she can remember. For the last ten years she had been followed up at our clinic, the last examination being in August 1978. The visual acuity in both eyes was 1.0 and the right pupil



Fig 4 (case 5)

Left eye of the 34 year-old woman with oval pupil since the age of 19

and iris were normal. The left pupil was oval and in the inferior temporal quadrant the pigmented epithelium of the iris was totally absent and some of the stroma was exposed. Gonioscopy revealed that the angle was open elsewhere but deformed by anterior retraction at the 3-6 o'clock position. Micrographs of the corneal endothelium showed that the cells of the two eyes were similar and the endothelial mosaic was regular.

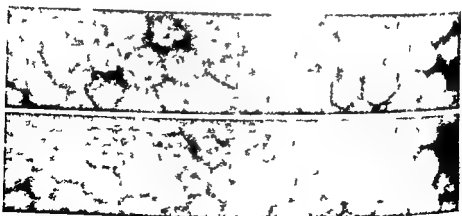


Fig 4A

Regular hexagonal endothelial cells of case No. 3. Top: left eye (9000 cells/mm<sup>2</sup>); Bottom: right eye (3000 cells/mm<sup>2</sup>) ( $\times 440$ )

*Table I*  
Signs and symptoms in the patients

Case No.	Diagnosis	Age (years)	Duration of disease (years)	Visual acuity in affected fellow eye	Highest VA in affected fellow eye (min/H)	Endothelial cells	Endothelial cell density (cells/mm <sup>2</sup> )	
							Affected eye	fellow eye
1	Essential iris atrophy	32	6	0/10	18/17	Abnormal	-	2800
2	Essential iris atrophy	42	> 3	10/10	40/46	Abnormal	-	3000
3	Essential iris atrophy	63	7	0/10	14/14	Abnormal	-	2500
4	Iridodochiae	60	13	10/10	1/13	Large	620	9000
5	Pupillary membrane (acquired)	34	1	10/10	1/1	Normal	2320	3000
6	Pupillary membrane (congenital)	38	congenital	10/10	14/13	Normal	3000	3000

## Discussion

The first three cases presented fulfil the criteria of typical essential iris atrophy in all respects. The disease began in an initially normal eye with mild distortion and displacement of the pupil. Next the pigment layer of the iris began to show defects. Finally large parts of the iris degenerated and features of secondary angle-closure destruction developed (Alkemade 1969). The description of Rieger's syndrome in the literature does not quite fit the findings made in any of these six patients. In Rieger's syndrome (dysgenesis mesodermalis of the iris and cornea) the primary dysgenesis is congenital rather than acquired, it is usually stationary rather than progressive and is always bilateral.

In eyes with essential iris atrophy the morphology and size of the corneal endothelial cells can be studied *in vivo* with the specular microscope. In the patients with essential iris atrophy (cases 1-3) specular micrographs show endothelial cells that are clearly abnormal morphologically and different from those of the normal fellow eyes. Hetherington (1978) found similar abnormalities in endothelial cells in patients with Chandler's syndrome, a probable variant of EIA. With the aid of electron microscopy Campbell et al. (1978) demonstrated aberrant endothelial cells over the angle and on the iris. The ectopic endothelial membrane has been detected in eyes that have been enucleated because of far advanced changes due to secondary glaucoma or in trabeculectomy and iridectomy specimens. In these previous studies the angle and trabeculum have been examined but not the endothelial cells of the central cornea.

In micrographs of normal corneal endothelial cells the cell surfaces reflect light and so appear light while the cell boundaries absorb light and appear dark. In essential iris atrophy the endothelial cells of the affected eyes reflect light abnormally, the cell boundaries being lighter and the cells darker. It is known that diseased endothelial cells form additional amounts of Descemet's membrane. Later the thickness of this membrane becomes uneven and the 'cobblestone pattern' is formed, which seems to account for the changes seen in the micrographs. Conceivably these corneal endothelial cells seem to oversecrete collagenous material in the same way as the cells in the chamber angle (Campbell et al. 1978).

The history and previous examinations of the three patients with essential iris atrophy revealed that the IOP was high in cases 2 and 3 but normal in case 1. The endothelial cell pattern was modified in a similar way in all three patients with EIA. In patients with glaucoma the IOP, although it affects the endothelial cell density, does not modify the appearance of the cells to the extent seen in these patients (Vannas et al. 1977; Setälä & Vannas 1978).

The fourth patient had iridoschisis and the cells, although very large, were

regular and sharply visualized. The low cell density may have been induced by the high IOP and the surgical trauma together.

The two last patients had stationary iridal disorders and do not fit in essential iris atrophy. The endothelial cells were normal in appearance in both eyes and the cell density did not differ between the affected and unaffected eye.

Essential iris atrophy is a rare monocular disorder which also involves the corneal endothelium. The pathological appearance of the endothelial cells calls for further studies with the specular microscope which can be used in differentiating essential iris atrophy from other iridal disorders.

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## CONTRACTILE PROPERTIES OF EXTRAOCULAR MUSCLE IN SIAMESE CAT

BY

Gunnar Lennerstrand

Siamese cats are albinos with poor visual resolution and severely impaired binocular vision. Eye muscle physiology was studied in Siamese cats as a part of a more extensive project on eye muscle properties in cats with deficient binocular vision. Isometric contractions of the inferior oblique muscle were recorded in response to single and repetitive muscle nerve stimulation. Speed of contraction, measured as twitch contraction time, fusion frequency and rate of tetanic tension rise, was lower in Siamese than in normal cats. Eye muscles of Siamese cats fatigued more easily to continuous activation than normal cat eye muscle. These functional changes have also been found in cats with binocular defects from monocular lid suture, but were much more marked in Siamese cats. It is suggested that the eye muscle changes represent muscular adaptations to genetically caused impairments of binocular vision and visual resolution in Siamese cats.

*Key words:* extraocular muscle - contractile properties - Siamese cats - binocular deficiency

Albinism is a genetic defect of melanin synthesis associated with impairment of visual function, such as low visual acuity, defective binocular vision and nystagmus (Duke Elder 1963). The neuroanatomical and neurophysiological basis for these visual abnormalities have been particularly well studied in the Siamese cats (Cline et al. 1974). Functional properties of retinal ganglion cells are different in normal and Siamese cats (Chino et al. 1978), but the most profound abnormalities in Siamese visual function can be related to a misrouting of a part of the retina.

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geniculate fibers (Guillery 1969 Guillery & Kaas 1971 Khalil et al 1971 Shatz 1977) These fibers project to the lateral geniculate body on the wrong side and this induces functional adaptations in the visual cortex (Hubel & Wiesel 1971 Cool & Crawford 1972 Kaas & Guillery 1973) as well as in structures like the pretectum and the superior colliculus that act as linkage between visual function and locomotion (Khalil et al 1971 Berman & Cynader 1972 Lane et al 1974) Recently Collewijn et al (1978) discovered a marked instability in the optokinetic responses of albino rabbits which they suggested was related to abnormal visuomotor connections The purpose of the present study was to investigate if these functional changes in Siamese cats might affect eye muscle properties

For normal binocular functions including stereopsis binocularly responsive visual cortex neurons are a prerequisite (Bishop 1973) In the Siamese cat all neurons in the visual cortex are monocularly driven (Hubel & Wiesel 1971 Cool & Crawford 1972 Kaas & Guillery 1973) and these cats have no stereopsis (Packwood & Gordon 1975) In pigmented cats with normal visual pathways similar changes in striate neuron function can be induced by permanent misalignment of the eyes if the cats have been squinting since they were four weeks of age or younger (Hubel & Wiesel 1965 Yinon 1976) Although convergent squint is of common occurrence in Siamese cats both the strabismic animals and the animals with apparently straight eyes show exactly the same abnormalities of cortical functions (Cool & Crawford 1972 Shatz 1977)

Disruption of binocular vision in normal cats by monocular visual deprivation at an early age was accompanied by changes in eye muscle function It was noted that the speed of contraction and the fatigue resistance were lower in the inferior oblique muscles of these animals than in the muscles of cats with normal binocular development (Lennerstrand & Hanson 1979) It will be shown in the present study that Siamese cats with a genetic defect of binocular vision exhibit the same kind of eye muscle abnormalities but to an even greater extent than the cats with acquired binocular dysfunction

## Methods

The experiments were performed on four Siamese cats one of which had extreme convergent strabismus but the rest were apparently non-squinting In all the cats the retina and the choroid completely lacked pigment

The animals were anaesthetized with pentobarbital (p (40 mg/kg b.w.) Previous papers by Hanson & Lennerstrand (1977) and Lennerstrand & Hanson (1978a, 1978b) have given detailed descriptions on (1) the preparation of the inferior oblique muscle for isometric tension recording to single and repetitive stimulation of the muscle nerve (2) the force-measuring system and (3) the stimulus procedures to study speed of contraction in twitch and tetanus fatigue properties and post-tetanic potentiation The rapid tension changes were

recorded on a storage oscilloscope (Tektronix 7103 A) and the responses with a slow rate course on an ink writer (Beskman Model RB) which was sufficient for resolving the tension changes during fatigue stimulations.

The results from six inferior oblique muscles in these four Siamese cats were compared with data from the same muscle in fully pigmented cats with no obvious impediments to normal binocular vision already reported by Lennertstrand & Hanson (1979). The means were used in the statistical analysis.

## Results

*Speed of contraction* was significantly lower in the inferior oblique muscles of the Siamese cats than in control animals. This was obvious from recordings of twitches as well as tetanic contractions. As shown in Table 1, twitch contraction time (*ct*) was 5.4 msec on an average in normal cats and 7.4 msec in the Siamese. The differences in the half relaxation (*hrt*) of the twitches were even larger: 7.1 msec in normals and 11.6 msec in Siamese cats. A typical twitch response of a Siamese inferior oblique is shown in Fig. 1. The contraction peak and the half relaxation point were reached considerably later than in normal cats (marked by arrows). As for the speed of tetanic contraction, significant differences were found between the groups in the rate of tension rise and the stimulus frequency to produce fused tetani (see Table 1).

The maximal twitch tension was higher in Siamese than in normal cats (5.4 and 3.7 g, respectively) but the maximal tetanic tension was not significantly different in the two groups.

On increasing the stimulus strength from threshold to supramaximal values the *ct* and *hrt* decreased steadily, i.e. the slowest contractions were recorded to threshold stimulation and the fastest to supramaximal stimulation. This is the same behaviour

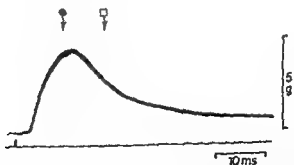


Fig. 1

Twitch contraction (top trace) to supramaximal nerve stimulation in a Siamese inferior oblique muscle. The lower trace carries the stimulus pulse. The arrows indicate peak of contraction (●) and half relaxation (□) of normal cat inferior oblique (see Lennertstrand & Hanson 1979). Twitch contraction time and half relaxation time are longer in Siamese than in ordinary cat eye muscles.



as seen in eye muscles of normal cats and probably means that threshold stimulation activated slow units in both Siamese and normal cats (see Lennerstrand & Hanson 1978a)

*Fatigue properties* were also markedly different in the two groups of cats. To continuous stimulation at 100 and 200 Hz the tension drop was much larger in the Siamese than in control cats as seen in Fig. 2. The tension remaining after 30 sec of stimulation in percent of the tension at stimulus initiation has been used as a measure on muscle fatigue resistance (endurance). As shown in Table I the average

Table I

Contractile characteristics in which significant differences existed between normal (N) and Siamese (Si) cats. Number of muscles (n) in each group as indicated. Mean values  $\pm$  SD are given. Twitch response parameters represented are amplitude (force difference between pre-twitch level and twitch peak), contraction time (ct) and half relaxation time (hrt) as indicated in Fig. 1. Tetanus response parameters presented are fusion frequency, i.e. the lowest stimulus frequency to reach a smooth or fused tetanus curve and rate of tension rise in the steepest part of the tetanus curve measured as percent of maximal tension ( $P_0$ ) per millisecond. Endurance has been measured as the tension output after 30 sec continuous stimulation at 100 or 200 Hz in percent of the tension at stimulus initiation (see also Fig. 2). Post tetanic twitch hrt in percent of pre tetanic values were determined for twitch responses obtained 5 min after a 30 sec tetanization at the frequencies of stimulation indicated.

	N (n = 8)	Si (n = 6)	P
<i>Twitch</i>			
ampl (g)	3.7 $\pm$ 1.0	3.4 $\pm$ 0.7	
ct (ms)	3.7 $\pm$ 0.6	4.4 $\pm$ 1.4	
hrt (ms)	7.1 $\pm$ 1.0	11.6 $\pm$ 3.9	
<i>Tetanus</i>			
fusion frequency (Hz)	331 $\pm$ 94	297 $\pm$ 95	
rate of tension rise (% $P_0$ msec)	1.8 $\pm$ 1.6	4.4 $\pm$ 1.4	
<i>Endurance (%)</i>			
at 100 Hz	95 $\pm$ 15	63 $\pm$ 13	
at 200 Hz	47 $\pm$ 4	39 $\pm$ 8	*
<i>Post tetanic twitch hrt (%)</i>			
at 50 Hz	103 $\pm$ 16	145 $\pm$ 32	
at 100 Hz	109 $\pm$ 10	172 $\pm$ 57	**
at 200 Hz	105 $\pm$ 29	186 $\pm$ 66	

0.05 < P < 0.01    0.02 < P < 0.01    \* P < 0.01

fatigue resistance was 94% at 100 Hz in normal cats while in the Siamese cats endurance was only 63%. At 200 Hz stimulation the values were 41% for the normal and 32% for the Siamese cats. During 50 Hz stimulations the tension of both Siamese and normal inferior oblique showed a steady increase thus displaying a so-called stair case phenomenon. In both groups final tension reached 100-140% of the initial value.

As a result of the long stimulations twitch responses showed progressive

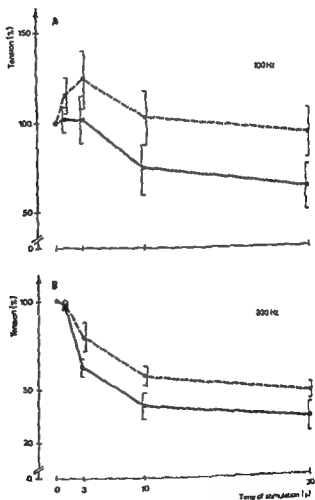


Fig. 2

Graphical display of tension development during stimulation at 100 Hz in A and 200 Hz in B. Values from Siamese cats are connected with full lines and those from normal cats with interrupted lines. Tension has been measured at 1, 3, 10 and 20 sec. of stimulation and expressed in percent of the tension at stimulus initiation. Average values have been plotted. Fatigue was more pronounced in Siamese than in ordinary cat eye muscle.

depression that lasted up to ten min and the  $m$  and  $hrt$  were prolonged (Lennerstrand & Hanson 1978b). These effects were quantitatively the same in normal and Siamese cats except for  $hrt$  which was significantly longer in Siamese twitches recorded 2 and 3 min after the fatiguing stimulus.

Responses to short tetanic stimulations (200 Hz for 0.5 sec) have been shown to be reduced for approximately two minutes as a result of continuous stimulation for 30 sec (Lennerstrand & Hanson 1978b). Similar effects were obtained in the Siamese group with no significant difference from the normal animals. Thus recovery of a tetanus after long muscle activation was equally rapid in both groups.

*Post-tetanic potentiation (PTP)* occurred after short tetani (200 or 400 Hz for 1 sec) as reported by Lennerstrand & Hanson (1978b) for normal eye muscles. The effects were the same in eye muscles of Siamese cats.

*Succinylcholine* is known to induce contracture in extrinsic muscle but flaccid paralysis in normal skeletal muscle (Bach & Rita 1971). Injections of 1 mg/kg b.w. produced a contracture of 10–15 min duration in both Siamese and normal cats. The peak tension obtained with the drug was 30–35% of maximal tetanic tension to nerve stimulation (at fusion frequency) in both groups of animals.

## DISCUSSION

Eye muscles of Siamese cats showed slower contractions and fatigued more easily than eye muscles in pigmented cats with normal binocular vision. The eye muscle abnormalities in Siamese cats were similar to, but more pronounced than those seen in pigmented cats reared monocular lid suture at an early age (Lennerstrand & Hanson 1979).

Siamese cats lack binocular vision (Packwood & Gordon 1975) probably as a result of a functional adaptation to a congenital aberration of the visual pathways. Part of the optic fibers from the temporal retina in the Siamese cats become misrouted and cross to terminate in the contralateral geniculate body; in normal cats these fibers reach the ipsilateral geniculate body (Guillery et al. 1974). Some Siamese cats have convergent squint but many have apparently straight eyes. However, squint was not correlated to the amount of functional adaptation in the visual cortex (Shatz 1977). The type of adaptation was either a suppression of the input from the ipsilateral eye (Hubel & Wiesel 1971) or a suppression of both the inflow from ipsilateral eye and from the temporal retina of the contralateral eye causing binasal visual field defects (Kaas & Guillery 1974; Guillery et al. 1974).

In addition Siamese cats showed deficits of contrast sensitivity and a lower visual resolution than normal cats (Blake & Antoinette 1976) which may be related to a retinal dysfunction due to albinism and lack of pigment in the retina.

Some defects of oculomotor functions have been reported in albinos in man. Spontaneous nystagmus often occurs from birth (Duke Elder 1963). The rabbits showed abnormal optokinetic responses (Collewijn et al. 1975). These signs of instability in the oculomotor control system might be related to the abnormalities of the visual pathways as suggested by the latter authors. However it is also possible that they were due to the eye muscle abnormalities discovered in this study, particularly the lowered fatigue resistance.

The eye muscle changes in Siamese cats might represent either a genetically determined alteration of eye muscle development or perhaps more likely particular adaptations to the congenital defects of binocular function and the lowered visual resolution. The eye motor system for binocular vision probably employs mainly the slow contracting and fatigue resistant fiber components found in eye muscles (Lennerstrand 1975; Lennerstrand & Hanson 1978b). In animals lacking binocular vision like the Siamese cats the demand for slow fusion vergence movements might be much reduced and this might lead to a retardation in slow component development. It has been suggested that similar mechanisms may induce the lowered fatigue resistance of eye muscles in normally pigmented cats with impaired binocular functions (Lennerstrand & Hanson 1979). Further the fast fiber components seem under-developed in Siamese cats. The visual resolution is low in these cats (Blake & Antoinette 1976) and the movement detection poor (Simon & Sprague 1976). This might result in a disuse of the saccadic system for fast reflexion movements which could then account for the reduced speed of eye muscle contraction in Siamese cats. It is also possible that the poor visual resolution curtailed the ability to maintain steady fixation and that due to the decreased demand for tonic fixational activity the slow eye muscle fiber system is affected. This would add to the defect that might be caused by the loss of binocular vision and explain why fatigue resistance of eye muscles in Siamese cats is still lower than that of pigmented cats with binocular defects but good visual resolution at least in one eye.

Thus both pigmented cats with acquired binocular impairment due to monocular visual deprivation and Siamese cats with a congenital defect of binocular vision showed marked changes of eye muscle function in comparison with the eye muscle properties of normal cats. Morphological and histochemical studies are in progress on the muscles examined physiologically. They indicate that the functional changes can be correlated with differences in the size of all types of muscle fibers and in the density of capillaries.

# Acknowledgment

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Table I

Indications and results of pars plana vitrectomy in 100 consecutive cases from 97 patients  
For definition of positive and negative result see text

	Total No of eyes	Results		No of patients	Average age (years)
		Positive	Negative		
Diabetic vitreous hemorrhage	97	26 (70%)	11 (30%)	95	51.2 (26-79)
Retinal detachment (vitreous hemorrhage secondary membranes vitreous retraction)	28	11 (39%)	17 (61%)	28	59.1 (7-80)
Vitreous hemorrhage due to various causes (branch vein occlusion, central vein occlusion, arterio- hypertension, diabetes mellitus, degeneration secondary to retinal detachment)	17	16 (94%)	1 (6%)	16	60.2 (37-71)
Vitreous hemorrhage due to trauma (pars plana surgery, contusion and mechanical foreign bodies)	13	6 (46%)	7 (54%)	13	51 (31-70)
Anterior segment surgery		1	1		64 (5-71)
Total eyes				97	
Total cases	100	43	57		
Success rate					54 (57%)

## Material and Methods

The material consists of 100 eyes from 97 patients with an average age of 53.1 years (7-60). Average follow up time was 14.2 months (1-38). Indications for vitrectomy and the number of patients in the different groups are shown in Table I.

Vitrectomies were performed with the Vitreous Stripper (Klout 1977a). The stripper is non rotating and cuts in an axial fashion. The first 40 cases were performed with the aid of the indirect ophthalmoscope but this method was abandoned as soon as an operation microscope (OPMI 6 Zeiss) was available since the microscope is easier and safer to use. The slit lamp mounted on the microscope has been the illumination source. All equipment used including corneal contact lenses has recently been described in detail by Klout (1977a).

## Results

Table I shows the results. A positive result was obtained in cases with a preoperative cloudy vitreous and a postoperative clear vitreous space (vitreous haemorrhages) and in eyes with retinal detachment the majority of the vitrectomies were technical successes but it was not always possible to reattach the retina. A positive result in these eyes means reattached retina. In the anterior segment group a positive result indicates that the aim of the operation was obtained. The change in visual acuity is illustrated in Fig. 1. Visual acuity was improved in 77%, unchanged in 24% and reduced in 21% on the latest examination in the follow up period.

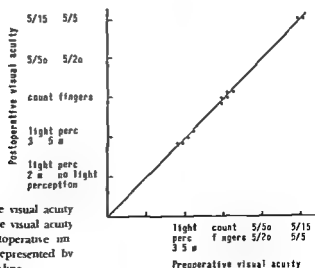


Fig. 1

Correlation of preoperative visual acuity (abscissa) with postoperative visual acuity (ordinate). Eyes with postoperative improved visual acuity are represented by dots above oblique line.

Table II

Summary of complications and important pre and postoperative factors in 100 consecutive cases of pars plana vitrectomy. In brackets the percent of each column. The total in each column exceeds the number in this column because one eye may be represented in more than one category of each column. The total in each column represents the failures in each group.

	Diabetic vitreous haemorrhage	Retinal detach	Vitreous haemorrhage var caus	Vitreous haemorrhage Trauma	Anterior segment vitrect	Total (%)
Total	37	23	17	13	5	100
Rebleeding, two or three vitrect	8 (21%) 4-	1 (4%) 1-		2 (15%) 1-		11 11-
Retinal tears		3 (11%) 1-		2 (15%) 2-		5 5-
Postop retinal detachment	1 (3%) 1-		1 (6%) 1-	5 (38%) 3-	1 1-	8 8-
Postop haemorrhage glaucoma	7 (19%) 7-					7 7-
1 top glaucoma control in medic	5 (8%) 0-					5 5-
Final heat during/ after vitrect	- ( )		1 (6%)	3 (3%)		4 4-
1 vitreal detachment	1-					1 1-
Post vitrect	1-					1 1-



Case	Sex	Age	Examination	Findings	Diagnosis	Prognosis	Remarks
1	F	10	Examination	0-	1 (10%)	1-	1 (10%)
2	F	10	Examination	3 (8%)	1 (10%)	1-	3 (8%)
3	F	10	Examination	0-	1 (10%)	1-	1-
4	F	10	Examination	13 (33%)	11 (31%)	1-	1-
5	F	10	Examination	1 (11%)	1 (11%)	1-	1 (11%)
6	F	10	Examination	0-	1 (11%)	1-	1-
7	F	10	Examination	0-	1 (11%)	1-	1-
8	F	10	Examination	14 (38%)	14 (38%)	1-	14 (38%)
9	F	10	Examination	4-	4-	1 (6%)	1 (6%)
10	F	10	Examination	3 (8%)	3 (8%)	0-	0-
11	F	10	Examination	0-	0-	0 (3%)	0-
12	F	10	Examination	0-	0-	0-	0-
13	F	10	Examination	0-	0-	0-	0-
14	F	10	Examination	0-	0-	0-	0-
15	F	10	Examination	0-	0-	0-	0-
16	F	10	Examination	0-	0-	0-	0-
17	F	10	Examination	0-	0-	0-	0-
18	F	10	Examination	0-	0-	0-	0-
19	F	10	Examination	0-	0-	0-	0-
20	F	10	Examination	0-	0-	0-	0-
21	F	10	Examination	0-	0-	0-	0-
22	F	10	Examination	0-	0-	0-	0-
23	F	10	Examination	0-	0-	0-	0-
24	F	10	Examination	0-	0-	0-	0-
25	F	10	Examination	0-	0-	0-	0-
26	F	10	Examination	0-	0-	0-	0-
27	F	10	Examination	0-	0-	0-	0-
28	F	10	Examination	0-	0-	0-	0-
29	F	10	Examination	0-	0-	0-	0-
30	F	10	Examination	0-	0-	0-	0-
31	F	10	Examination	0-	0-	0-	0-
32	F	10	Examination	0-	0-	0-	0-
33	F	10	Examination	0-	0-	0-	0-
34	F	10	Examination	0-	0-	0-	0-
35	F	10	Examination	0-	0-	0-	0-
36	F	10	Examination	0-	0-	0-	0-
37	F	10	Examination	0-	0-	0-	0-
38	F	10	Examination	0-	0-	0-	0-
39	F	10	Examination	0-	0-	0-	0-
40	F	10	Examination	0-	0-	0-	0-
41	F	10	Examination	0-	0-	0-	0-
42	F	10	Examination	0-	0-	0-	0-
43	F	10	Examination	0-	0-	0-	0-
44	F	10	Examination	0-	0-	0-	0-
45	F	10	Examination	0-	0-	0-	0-
46	F	10	Examination	0-	0-	0-	0-
47	F	10	Examination	0-	0-	0-	0-
48	F	10	Examination	0-	0-	0-	0-
49	F	10	Examination	0-	0-	0-	0-
50	F	10	Examination	0-	0-	0-	0-
51	F	10	Examination	0-	0-	0-	0-
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57	F	10	Examination	0-	0-	0-	0-
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65	F	10	Examination	0-	0-	0-	0-
66	F	10	Examination	0-	0-	0-	0-
67	F	10	Examination	0-	0-	0-	0-
68	F	10	Examination	0-	0-	0-	0-
69	F	10	Examination	0-	0-	0-	0-
70	F	10	Examination	0-	0-	0-	0-
71	F	10	Examination	0-	0-	0-	0-
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74	F	10	Examination	0-	0-	0-	0-
75	F	10	Examination	0-	0-	0-	0-
76	F	10	Examination	0-	0-	0-	0-
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79	F	10	Examination	0-	0-	0-	0-
80	F	10	Examination	0-	0-	0-	0-
81	F	10	Examination	0-	0-	0-	0-
82	F	10	Examination	0-	0-	0-	0-
83	F	10	Examination	0-	0-	0-	0-
84	F	10	Examination	0-	0-	0-	0-
85	F	10	Examination	0-	0-	0-	0-
86	F	10	Examination	0-	0-	0-	0-
87	F	10	Examination	0-	0-	0-	0-
88	F	10	Examination	0-	0-	0-	0-
89	F	10	Examination	0-	0-	0-	0-
90	F	10	Examination	0-	0-	0-	0-
91	F	10	Examination	0-	0-	0-	0-
92	F	10	Examination	0-	0-	0-	0-
93	F	10	Examination	0-	0-	0-	0-
94	F	10	Examination	0-	0-	0-	0-
95	F	10	Examination	0-	0-	0-	0-
96	F	10	Examination	0-	0-	0-	0-
97	F	10	Examination	0-	0-	0-	0-
98	F	10	Examination	0-	0-	0-	0-
99	F	10	Examination	0-	0-	0-	0-
100	F	10	Examination	0-	0-	0-	0-

A summary of factors of importance pre and postoperatively are listed in Table II. The percentages in table and text are calculated to make the groups comparable.

**Diabetic vitreous haemorrhage** Preoperative visual acuity was less than for counting at 1 meter in all eyes. Twenty two of 37 eyes (59%) had visual improvement of one degree after vitrectomy. Vitrectomy had to be repeated in eight eyes due to rebleeding. Postoperative haemorrhagic glaucoma developed in seven eyes and resulted in enucleation in two eyes. One eye ended amniotic because of postoperative endophthalmitis. In two eyes where the corneal epithelium was removed after vitrectomy very slow healing took place.

Lens opacities were frequent in this group. Lens extraction prior to vitrectomy for optical reasons, lensectomy during vitrectomy and postoperative lens extraction due to increasing cataract made a total of 20 eyes (54%) aphakic. Fourteen eyes had been treated with photocoagulation prior to vitrectomy and 10 of these eyes ended with a positive result.

**Retinal detachment** Vitrectomy was performed because of vitreous opacities (11 eyes), pupillary membranes (6 eyes) and vitreous retinal traction (9 eyes). Retinal detachment was secondary to intracapsular cataract extraction with vitreous loss in seven eyes, two eyes had uncomplicated extracapsular extractions and in one eye the lens had been removed because of subluxation after ocular contusion. An accidental lens injury happened in one eye during vitrectomy leading to lensectomy. Accidental retinal tears occurred in three eyes. Retinal detachment surgery after vitrectomy was performed on all eyes except one which was considered inoperable due to massive periretinal retraction (MPR).

**Vitreous haemorrhage of various causes** The underlying cause of vitreous haemorrhage was central vein occlusion (1 eye), branch vein occlusion (8 eyes), pathologic photocoagulation mechanism (1 eye), arterial hypertension (2 eyes), disciform macular degeneration (2 eyes) and raised intracranial pressure during subarachnoid haemorrhage (3 eyes). The interval from thrombosis to vitrectomy varied from 1–21 years (average 9.7 years) and non-resorbing haemorrhage had in these eyes been present from 2 months – 15 years (average 22.1 months). One eye with disciform macular degeneration was melanoma suspect and the eye was enucleated on suspicion when the fundus was seen during vitrectomy. The histology showed disciform macular degeneration and vitreous bleeding (prep. No. 1346/71). One eye developed retinal detachment and was lost due to MPR.

**Vitreous haemorrhage caused by trauma** The underlying cause was ocular contusion (2 eyes) perforating injuries (7 eyes) and intraocular non magnetic foreign bodies (4 eyes). One foreign body was removed by forceps, three could not be removed and the eyes were enucleated to avoid the risk of sympathetic ophthalmia. Three of seven eyes with perforating injuries were not successful due to retinal detachment and VPR. In two eyes, retinal detachment developed two months after vitrectomy, which ended successfully after retinal detachment surgery. Two eyes ended as failures due to retinal tears produced during vitrectomy.

**Anterior segment** Corneal decompensation due to vitreous touching the corneal endothelium induced vitrectomy in four eyes. One eye had phacolytic glaucoma, and lens remnants and vitreous were removed. These eyes were technical successes but one eye developed a postoperative retinal detachment and retinal surgery was not successful.

### Discussion

Vitrectomy gave a positive result in 63% and visual improvement in 55% of our first 100 cases. Michels & Ryan (1975) found visual improvement in 64% and Peyman et al (1976) in 68% of their first 100 cases. Results on 400 cases are reported (Peyman et al 1978) and results are also published on diabetic eyes (Mandelcorn et al 1976, Michels 1978, Blankenship & Machemer 1978) and on the management of traumatic cases (Hutton et al 1976, Benson & Machemer 1976, Conway & Michels 1978). Complications are reported by Machemer (1972), Klotz (1977b), Tardif & Schepens (1977), Puhlhorn et al (1977), Perry et al (1978).

Complications of diabetes is the most frequent indication for vitrectomy. Even if complications in vitrectomy are frequent in this group, visual improvement is the result in a high percentage (59% present material, 60.8% Michels & Ryan 1975, 68% Peyman et al 1976, 53% Mandelcorn et al 1976, 66% Peyman et al 1978, 65% Michels 1978).

In the diabetic group, lens extractions were performed for optical reasons but also in order to remove the barrier between the vitreous cavity and the filtration system. Blood in the vitreous cavity is in this way more easily absorbed. This view is supported by the result in the present series where 80% of the aphakic eyes (17 out of 21) ended with a positive result compared to 53% of the phakic eyes (9 out of 17).

Corneal complications occurred in two eyes, both diabetic. Peyman et al (1978) found corneal oedema and striae in 38% in the early postoperative period and as a complication in 7%. Perry et al (1978) found that corneal changes developed in 13%. Diabetic patients have a poor adhesion of the corneal epithelium (Mandelcorn et al 1976) and the greatest risk for postoperative corneal problems. Many

surgeons do routinely remove the corneal epithelium during vitrectomy. In the present series this has not been performed except in the two diabetic eyes and the corneas stayed clear during the operation. The experience from this series of eyes does therefore not support the view that it is necessary to remove the corneal epithelium. On the contrary this may induce postoperative corneal complications.

As a total retinal tears occurred in 5% in the present material. Figures vary from 37% (Michels & Ryan 1975) to 2% (Peyman et al 1978). In eyes with complicated retinal detachment Peyman et al (1978) had a success rate of 49%. 39% of the eyes in the present series ended reattached. All eyes were however considered to be rather hopeless without vitrectomy and the result must be seen in view of these circumstances.

46% of our traumatic cases ended with visual acuity improved compared to 31% in Benson & Machemer's (1976) series, 64% by Hutton et al (1976) and 60% in Conway & Michels (1978) series. The best results are obtained where vitrectomy is performed within 1-2 weeks after the injury.

Ora dialysis (Machemer 1972 6.6%) and fibrous ingrowth from the scleral opening (Tardif et al 1977 11.2%) have not been seen in the present material. The pilot tube for the Stripper is considered important in the prevention of these complications.

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## ULTRASONIC MEASUREMENTS OF THE EYE IN THE NEWBORN INFANT

BY

SVEN BLOMDAHL

The right eyes of 28 newborn infants have been examined reinoscopically and the anterior chamber depth, the lens thickness and the length of the vitreous were measured by ultrasound. In 15 of the infants keratometry was performed using the Javal Schiotz keratometer. The total axial length of the eye was found to be longer the heavier the weight of the baby, whereas the lens thickness showed no correlation to the total axial length or to the weight of the baby. In only 1/3 of the babies was the corneal astigmatism less than 0.5 D. A significant correlation ( $P < 0.01$ ) was found between the total axial length of the eye and the length of the corneal radius.

**Key words:** corneal radius - astigmatism - lens thickness - ultrasound - infants - axial length

Ultrasound biometry has recently been used for determining the axial length of the eye in the newborn child as well as the depth of the anterior chamber, lens thickness and the length of the vitreous body (Gernet 1964, Luyckx 1966, Grzes & Rivara 1968, Larsen 1971). A survey of their values is presented in Table I.

Previously, the axial length of the eye in the newborn infant had to be measured on cadavers (von Jäger 1861, von Pflugk 1909, Sorsby & Sheridan 1960).

The aim of the present study has been to investigate the possible relationships between the weight of the newborn baby, the axial length of the eye, lens thickness, the corneal radius and the refraction. To this aim the axial depth, the anterior chamber, lens thickness and vitreous length have been measured by ultrasound in newborn babies. Reinoscopy has been performed and in some cases also keratometry using the Javal Schiotz keratometer.

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*Table I*

Earlier determinations of the axial values of the eye in infants

	Larsen		Gernet		Luyckx		Grignolo
	F	M	F	M	F	M	F + M
ant. chamber (mm)	2.39	2.37	2.9	2.9	2.5	2.6	-
lens (mm)	3.99	3.93	3.4	3.4	3.6	3.7	-
vitreous (mm)	10.22	10.48	-	-	10.8	10.8	-
tot. ax. length (mm)	-	-	17.2	17.1	17.7	17.5	17.02
refraction (D)	-	-	+2.6	+3.0	+2.2	+2.6	+0.52
infants examined (n)	37	43	15	21	25	27	18
eyes examined (n)	74	86	29	41	50	54	36
cycloplegic	-	-	Atropin		Cyclogyl		Tropamide

### Material and Methods

Twenty-eight healthy fullterm infants 14 girls and 14 boys have been examined. Their ages ranged from 1-4 days and their weights from 2910 to 4790 g. The eyes to be examined were made cycloplegic by 2 drops of 1% cyclopentolate hydrochloride (Cyclogyl®) instilled twice in the conjunctival sac. 40 min later the examination started. After local anesthesia with 0.2% oxubuprocaine chloride (Novesin®) a speculum not causing any pressure upon the bulb was inserted to keep the lids apart. The infants were held in an upright position by an assistant. Their heads were put on the chin and forehead rest of the Javal Schiotz keratometer. The examiner could easily see the reflexes on the cornea and repeated examinations in different meridians were performed. In 15 babies everyone quite peaceful the reflexes could be seen on the central cornea and 3-5 repeated examinations showed unanimous low measuring values. In 13 babies it was impossible to centralize the reflexes on the cornea. With the baby lying down the horizontal corneal diameter was measured using a caliper.

Retinoscopy was performed in two axes and finally the ultrasonic biometry could take place. This was done with an A-scan apparatus the Kretztechnik 7200 MA and with a transducer frequency of 8 MHz. This instrument is equipped with an electronic scale and a built in quartz oscillator which makes calibration possible previous to each examination. A contactglass similar to that described by Jansson (1963) and Pallin (1969) was used with an outer opening of 12 mm large enough not to press on the cornea during the examination as the corneal diameters were all less than 11 mm. The distance between the transducer and the cornea was 15 mm and this part of the contactglass was filled with saline easily refilled (if needed). The baby was lying down during the examination with the head kept steady by an assistant. When the echogram on the screen showed maximal amplitudes from the

reflecting surfaces a polaroid picture was taken. The distance in metres to the reflecting surfaces could then be converted into millimetres according to the velocities given by Jansson (1963) 1532 m/sec in the vitreous and aqueous and 1641 m/sec in the lens at 37°C. Both eyes were measured by ultrasound in 10 of the infants in order to find out if there were any measurable differences between the right and the left eye.

## Results

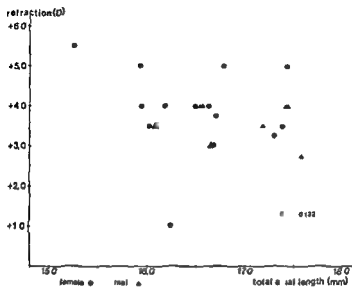
**Retinoscopy.** The range of the retinoscopic readings was from +1.0 D to +5.5 D with an average of +3.0 D. The girls had an average of +3.9 D and the average of the boys was +3.4 D. Sixteen of the 28 infants showed no astigmatism, nine had an astigmatism with the rule and three infants had an astigmatism with oblique axes.

Table II

The retinoscopic findings in two axes, values of the corneal radius in two axes and the corneal power in diopters of the corneas in two axes (calculated from the corneal radius)

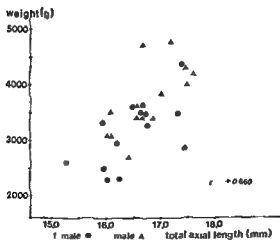
infant nr	retinoscopy(D)	corneal radius(mm)	corneal power(D)
1	+3.0	+4.5	+4.1
2	+4.0	+3.05	+4.1
3	+1.0	+4.5	+4.1
4	+3.5	+2.5	+4.1
5	+2.5	+2.7	+4.5
6	+0.5	+5.5	+2.5
7	+1.0	+4.0	+4.1
8	+4.0	+2.5	+4.1
9	+2.0	+2.5	+4.5
10	+3.0	+2.1	+4.1
11	+4.0	+2.7	+4.1
12	+0.5	+5.5	+2.5
13	+3.0	+2.7	+4.5
14	+4.0	+2.7	+4.1
15	+4.0	+4.5	+4.1





*Fig 1*

The refractive value plotted against the total axial length of the eye in 28 infants



*Fig 2*

The weight of 28 infants plotted against the total axial length of the eye

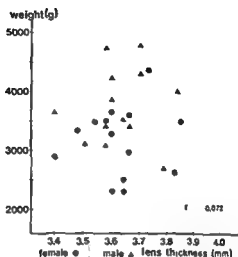


Fig 3

The weight of 28 infants plotted against the total axial length of the lens.

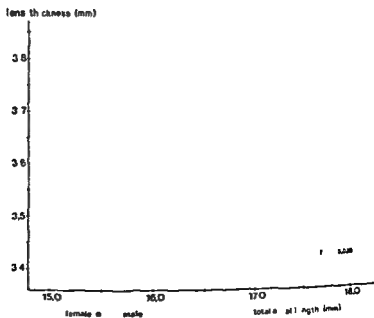


Fig 4

The lens thickness plotted against the total axial length of the eye in 28 infants.

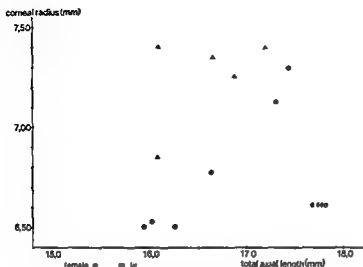


Fig. 5

The corneal radius plotted against the total axial length of the eye in 13 infants

The highest astigmatism was 4 D with +6 D in the horizontal and +2 D in the vertical meridian

**Ultrasound measurements** The depth of the anterior chamber in the 28 children ranged from 2.4 to 2.9 mm. The average value was 2.6 mm. The central thickness of the cornea was included in these readings.

The lens thickness values ranged from 3.4 to 3.9 mm with an average of 3.6 mm. The axial vitreous length ranged from 8.9 to 11.2 mm with an average of 10.4 mm. The total axial length ranged from 15.3 to 17.6 mm with an average of 16.6 mm. The mean value of the boys was 16.7 mm and 16.5 mm of the girls. In the ten babies where both eyes were measured by ultrasound the difference of the axial lengths never exceeded 1.2 mm.

**Keratometry** In the 15 babies examined with keratometry the range of the corneal radius was from 6.4 to 7.4 mm with an average of 7.0 mm. In five of the babies there was no astigmatism to be found and five babies had an astigmatism with the rule ranging from 1–4 D. Five babies showed an oblique astigmatism ranging from 1–2 D. Table II shows the values of the retinoscopic figures of the corneal curvature and of the corneal power in the same eye. In two of the infants with oblique corneal astigmatism this had not been registered by retinoscopy.

**Corneal diameter** The values of the corneal diameter in the horizontal plane varied from 9.0 to 10.5 mm with an average of 9.8 mm.

**Correlations** Fig. 1 shows the association between the refraction and the total axial length of the eye. The correlation coefficient is  $-0.132$  and there is no significant correlation between the degree of hypermetropia and the axial length of the eye ( $P > 0.1$ ) in this group.

Fig. 2 is a graph of the association between the weight of the baby and the total axial length of the eye. The correlation coefficient is  $0.660$  a value that differs from zero ( $P < 0.001$ ) showing that the heavier child has a longer eye.

Fig. 3 shows the relation between the weight of the baby and the lens thickness. The correlation coefficient for these parameters is  $0.072$  a value quite close to zero ( $P > 0.1$ ) and there is no correlation.

Fig. 4 shows the association between the lens thickness and the total axial length of the eye. Again the correlation coefficient is close to zero ( $0.019$ ) and these parameters are not significantly correlated ( $P > 0.1$ ).

In Fig. 5 the corneal radius is plotted against the total axial length of the eye in 15 infants. The value of the corneal curvature radius is given as the average value of the main meridians. The correlation coefficient of the parameters is  $0.614$  which significantly differs from zero ( $P < 0.01$ ). This suggests that the longer the total axial length of the eye the longer the corneal radius i.e. the flatter the cornea.

## Discussion

The refractive values of new borns found by other investigators mostly lie between  $+2$  D to  $+3$  D (Duke Elder 1969). In the 28 babies of this study the values varied from  $+1.0$  D to  $+5.5$  D and the average value of  $+3.6$  D is somewhat higher than that normally found.

Luyckx (1966) and Larsen (1971) using a contact glass between the eye and the transducer found the anterior chamber to be about  $2.4$  and  $2.6$  mm. The central thickness of the cornea was included in these values. Gernet (1964) found a value of  $2.9$  mm of the anterior chamber (still including the central corneal thickness). This value however can not be compared to the value found by the former authors because Gernet did not use a contact glass between the transducer and the eye and had to reckon with a pressure on the cornea in his calculations.

The mean values of the lens thickness recorded from other ultrasonic studies vary from  $3.4$  mm to  $4.0$  mm and the values of the vitreous length from  $10.9$  mm to  $10.8$  mm (Table 1). The oculometric mean values of the present study are all in accordance with these figures.

The average of the total axial length is  $16.6$  mm slightly less than the value obtained by Gernet, Luyckx and Grignolo but close to the average value  $16.1$  mm from Larsen's studies.

Luickx and Gernet found no relationship between the refraction and the axial length of the eye—neither could this be found in the present study. This is rather unique keeping in mind that refraction and axial length are so strongly correlated in all other age-classes. The finding implies that in the smaller eye the refractive power of the cornea and— or the lens must be stronger than in the longer eye.

In emmetropic eyes of adults Sorsby et al (1957) found a good correlation between the axial length and the corneal power. Fledelius (1976) found a significant correlation between these parameters in children aged about ten years. This study shows a significant correlation between the total axial length of the eye and the corneal power, and the longer the total length of the eye the smaller is the corneal refractive power. Thus the cornea compensates for the high degree of hyperopia which otherwise would be expected. Changes in lens power might also be compensatory, but the present investigation shows no correlation between the lens thickness on one side and refraction or axial length on the other.

Keratometry performed on stillborn infants in the early 20th century showed a corneal radius of about 7.0 mm (de Vries 1901, von Pflugk 1909). In two living infants Gernet (1964) found a radius of 6.4 mm and 7.7 mm using a Zeiss ophthalmometer. Grignolo & Rivara (1968) measured the corneal curvature in premature newborns, fullterm newborns and children to the age of ten. They used the Javal ophthalmometer and when this was not possible the measurements were carried out by juxtaposition of concave stencils representing arcs of circles with known curvature radii. They found the corneal power to be +5.3 D in fullterm newborns, a value that equates with a radius of about 6.3 mm. All considered the mean value 7.0 mm of the corneal radius from the present study seems to be close to the mean value of earlier investigators.

There seem to be no measurements of the astigmatism of the cornea carried out in living newborn infants. Marin Amat (1956) expected most corneas to be spherical. This assumption was made on the fact that in children of the age of three 50% had corneal astigmatism and at the age of seven 90% had corneal astigmatism. They therefore found it probable that in newborns the astigmatism approximately was zero. The present investigation does not favour this theory: 66% of the fifteen babies having astigmatic corneas. It must be pointed out, however, that the material is limited.

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# VILLOUS PROCESSES FROM THE INNER SEGMENTS OF CONES IN THE HUMAN MACULA LUTEA

BY

MARTIN DAVANGER and AMUND RINGVOLD

The cones of the human macula lutea were examined by scanning electron microscopy. It was found that each inner segment had about 15 thread like processes which projected from the cell surface. They were arranged in a longitudinal straight line along the photoreceptor and were directed away from or towards the fovea centralis. The villous processes may be analogous with the lateral fins of the inner segments of certain vertebrates. They perhaps bridge the space between neighbouring photoreceptors and establish contact and interaction between them.

*Key words:* retina—macula lutea—cones—inner segments

During the fresh specimen dissection of the posterior part of a normal eye ball the retina frequently becomes detached. The two surfaces created, the outer segments of the photoreceptors and the villous apical surface of the pigment epithelium, present themselves for scanning electron microscopy (SEM). However, in some specimens the adherence between the two layers may be stronger. Factors influencing this adherence have been studied, and among other things it has been shown that the adherence is stronger *in vivo* than *in vitro* (Zauberman et al. 1972; Zauberman & Guillebon 1972). Part of this adherence may be created by leaf-like processes of the pigment epithelium which ensheath the cone outer segments (Hogan et al. 1974; Steinberg & Wood 1974; Steinberg et al. 1977). Clinical experience with retinal detachment indicates that the adherence is stronger in the macular region than in the fundus in general.

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The specimens were dehydrated in graded acetone solutions and dried by the critical point method in fluid CO<sub>2</sub> (Sorvall Critical point drying system). The dried specimens were mounted on specimen holders and coated with a thin layer of carbon and gold palladium in a Edwards vacuum coating unit. Scanning electron microscopy was performed with a Jeol JSM 50 SEM.

## Results

In the specimen from the neuro retina it was found that in large parts of the parafoveal area most of the outer segments of the cones had been torn from the inner segments at the connecting cilium. This could be confirmed by the examination of the specimen containing the pigment epithelium. In the fovea itself the inner segments had also been removed during the preparation (Fig. 1).



Fig. 2

Cone inner segments from the parafoveal region displaced by the outer segments being removed during the preparation. Numerous thread-like processes project from the cell surface. On each photoreceptor they are arranged in a longitudinal straight line and they are directed away from or towards the fovea. SEM  $\times 5600$ . Bar =  $5\mu\text{m}$ .



Fig. 3

Trifoveal cone inner segments with processes probably cellular villi. Arrow points to a process which seems to bridge between two neighbouring photoreceptors.

Bar = 1  $\mu\text{m}$

The inner segments appeared as slightly conical elements, the outer end tapered gradually off into a thin extension where the cilium was located. A few remaining outer segments were found. During the preparation the inner segments had been running parallel, forming a sharp angle with the retina, 1–2  $\mu\text{m}$  apart, separated from each other by an empty space.

An unexpected observation was made when the inner segments were studied with higher magnification (Fig. 4).

processes measuring up to 2  $\mu\text{m}$  in length protruded about perpendicularly from the cell surfaces. Their diameter was slightly less than 0.1  $\mu\text{m}$ , remarkably constant both along the single process and from one process to the other. They were situated along one straight line from near the cilium and extending usually as far as the inner segment could be seen. The distance between the processes was rather uniform, about 1/3  $\mu\text{m}$ . Typically, one photoreceptor had about 15 processes.

The processes were directed either towards the fovea or away from it; the few exceptions were connected with cells which probably had been twisted during the preparation. The cell surface seemed to go directly into the surface of the processes with no discontinuity. Most of the processes ended in the intercellular spaces. Arrangement caused by the processing made it difficult to determine with certainty whether the processes *in vivo* had formed bridges between the cells. A search for similar processes outside the parafoveal area has been negative.

## Discussion

The appearance of the single process and the pattern they form together indicate that they represent real histological structures. Although their nature cannot be fully determined by SEM alone, we suggest that they are cellular villi, and to our knowledge, similar findings have not been described before. In the following, the structures on which we are reporting will be called the lateral processes of the inner segments.

It should be noted that three other kinds of cellular extensions or villi are present in this region of the human eye (Fig. 4): 1) Villous processes extend from the apical surface of the pigment epithelial cells to surround the photoreceptors' outer segment. 2) Villi forming the so-called fibrillar basket project from the Müller cells to the intercellular space between the inner part of the inner segment. 3) The inner segments themselves have a number of cytoplasmic extensions, the so-called filical processes (Cohen 1961) projecting outwards from the outer end of the inner segments and surrounding the basal quarter of the outer segments. The lateral processes on which we are reporting may be misinterpreted in transmission electron microscopical studies as being profiles of one of the cellular extensions mentioned. The nature of the lateral processes and their regular distribution is best recognized by scanning electron microscopy.

The occurrence in other species of lateral processes of the inner segments or similar structures remains to be determined. The lateral processes may perhaps be analogous with the lateral fins of the inner segments of some lower vertebrates (fishes, reptiles, birds) (Dunn 1973). These processes are radial, fin-like extensions of the plasma membrane, extending laterally from the body of the inner segment.

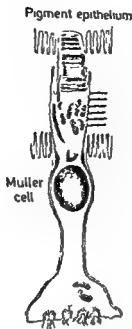


Fig. 4

Schematic drawing of a cone with adjacent pigment epithelial cell and Muller cell. The villous processes from these cells are emphasized. 1 Villous processes of the pigment epithelium. 2 The fibrillar basket villi of the Muller cells. 3 The calycal processes of the inner segment surrounding the basal quarter of the outer segment. 4 The lateral processes of the inner segment.

and imparting a gear like shape to a cross section of the visual cell. The lateral processes described in the present work may perhaps represent ventral or lateral fin remaining only in the form of these processes.

Nothing decisive can be said about the function of the lateral processes of the inner segments. The following possibilities may be considered:

- 1) Other cytoplasmic extensions from the inner segments (the calycal processes (Fig. 4) and the lateral fins mentioned above) and also the fibrillar basket extensions of the Muller cells (Fig. 4) have been presumed to play a role in the uptake of nutrient material from the extracellular space (Dunn 1961). A similar function may be ascribed to the lateral processes.
- 2) It has been asserted that the intercellular matrix between the photoreceptors and the pigment epithelium serves as an adhesive between these two layers (Zimmerman & Eastham 1959). All processes projecting from these cells into the matrix will promote this effect.

3) Neighbouring photoreceptors are separated by a space measuring 1–2  $\mu\text{m}$ . Up to now no anatomical contact has been demonstrated between human photoreceptors outside the external limiting membrane. Although it is difficult to determine with certainty whether the lateral processes have formed bridges between the photoreceptors *in vivo*, this may have been the case.

Extensive interactions between photoreceptors in toads have been found by physiological experiments and evidence has been presented indicating that the basis for this interaction is the presence of gap junctions between the lateral fins of adjacent photoreceptors (Fam et al. 1976).

On this background it may be presumed that the physiological role of the lateral processes of the inner segments is to establish contact and interaction between neighbouring photoreceptors. The regular distribution and radial direction relative to the fovea centralis of the lateral processes may be considered on the basis of this concept of their function.

As said above the villous processes undoubtedly represent real anatomical structures. However as indicated by the conical configuration of the inner segments (Figs 2 and 3) the separation of the retina from the subjacent pigment epithelium by tearing is obviously a rather rough procedure which probably leads to some form changes of the cells. In the further studies of these structures therefore a different preparation technique will be used.

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# MORPHOLOGY AND ENZYME ACTIVITIES OF THE RETINAL CAPILLARIES IN STREPTOZOTOCIN DIABETIC MICE

BY

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Male lean mice belonging to the obese hyperglycemic strain were made diabetic by intravenous injection of streptozotocin. The retinal capillary bed freed by trypsin digestion was studied with regard to morphology and the activity of some enzymes. There was a significant increase in the ratio between the endothelial and mural cells which was interpreted as indicating mural pericyte disappearance. The activities of adenylate kinase, aspartate amino transferase and hydroxyacyl CoA dehydrogenase in the retinal vessels of the diabetic animal were significantly higher than in vessels from the control animals. No differences were found in the activities of glucose 6-phosphate dehydrogenase, glutathione reductase and phosphofructokinase between the two animal groups.

It is suggested that these results reflect early morphological and metabolic changes of the retinal vessels preceding the well known clinical picture of diabetic retinopathy.

*Key words:* retinal diabetic retinopathy - capillary metabolism - capillary enzyme activities - mice

Many animal models have been used for experimental studies in diabetic retinopathy. Recently mice with the obese hyperglycemic syndrome which are characterized by marked hyperglycemia and hyperinsulinemia have been studied for possible retinal changes (Näeser & Ågren 1978). No obvious changes in the morphology of the capillary bed were seen. However, the activities of the enzymes adenylate kinase, aspartate aminotransferase and hydroxyacyl CoA dehydrogenase were higher in the obese mice than in the lean non-diabetic controls.

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The aim of the present investigation was to study the capillary bed in experimental diabetes with low insulin values and to compare the results with those of the obese hyperglycemic mice. For this purpose mice with diabetes induced by streptozotocin were selected.

## Materials and Methods

In all 127 male lean mice belonging to the obese hyperglycemic strain which has been bred at the Department of Histology, Uppsala, Sweden since 1959 (Wessler 1968) were used. The mice were kept in plastic cages and allowed free access to pelleted food and water (Vessler 1973). At the age of 2 months diabetes was induced by a single injection of streptozotocin (120 mg/kg body weight) into the tail vein. The streptozotocin was kindly placed to our disposal by Dr W. E. Dunn, Upjohn Co., Kalamazoo, USA. Two months after the induction of diabetes the animals were killed and the eyes were rapidly removed and placed in 4% formaldehyde solution for 2 h. Then the eyes were further processed for morphological and enzymatic determinations as described previously (Vessler & Ågren 1978). At the time of killing serum glucose was determined from all animals by a glucose oxidase method (Hjelm & deVerdier 1963). For statistical evaluation Student's *t* test was used (Snedecor 1967).

## Results

Altogether 77 mice were given streptozotocin injections. Fifty non-injected lean litter mates served as controls. At the time of death the serum glucose was determined. Fifty-five mice with serum glucose values above 15 mmol/l (500 mg per 100 ml) were used. The remaining mice had glucose values below 15 mmol/l and were not considered diabetic. The mean serum glucose concentration of the diabetic animals was  $31.1 \pm 1.3$  mmol/l and differed significantly from the serum glucose of the control animals ( $8.7 \pm 0.2$  mmol/l,  $P < 0.001$ ).

The ratio of endothelial cells to mural cells was examined on glass-microtome prepared mounted preparations of the retinal vessels. In the streptozotocin diabetic mice the ratio was significantly higher ( $4.82 \pm 0.17$ ,  $n = 8$ ) than in the control animals ( $3.43 \pm 0.02$ ,  $n = 11$ ,  $P < 0.001$ ). Mesodermal intercapillary bridges were present in all the preparations. In the preparations obtained from the streptozotocin diabetic mice the bridges occasionally showed elongated dark nuclei similar to those of mural cell nuclei. There were no areas showing acellularity or degeneration.

The results of the enzymic activities are given in Table I. It can be seen that the



Table I  
Enzyme activities of the retinal capillaries in streptozotocin diabetic mice

Enzyme	Streptozotocin injected	Non injected	Probability
Adenylate kinase EC 2.7.4.3	18.5 ± 6 (n = 13)	8.5 ± 4 (n = 10)	P < 0.001
Aspartate aminotransferase EC 2.6.1.1	47 ± 2 (n = 10)	2.0 ± 0.9 (n = 11)	P < 0.001
Glucose-6-phosphate dehydrogenase EC 1.1.1.49	1.0 ± 0.2 (n = 4)	0.9 ± 0.1 (n = 4)	NS
Glutathione reductase EC 1.6.4.2	0.51 ± 0.07 (n = 8)	0.46 ± 0.06 (n = 8)	NS
Hydroxyacyl-CoA dehydrogenase EC 1.1.1.35	2.2 ± 0.1 (n = 14)	1.6 ± 0.1 (n = 11)	P < 0.001
Phosphofructokinase EC 2.7.1.11	4.0 ± 0.1 (n = 9)	4.3 ± 0.1 (n = 11)	NS

All values are expressed as mmol/kg dry weight and hour and represent mean values ± SEM. Number of animals are given within parentheses. The experimental animals were lean mice from the obese hyperglycemic strain.

activities for adenylate kinase, aspartate aminotransferase and hydroxyacyl-CoA dehydrogenase differ significantly from the controls. The activities for glucose-6-phosphate dehydrogenase, glutathione reductase and phosphofructokinase did not however differ significantly between the groups.

## Discussion

Decrease in the number of mural pericytes in diabetic retinopathy has been reported in human diabetes (Cogan et al 1961; Kuwabara & Cogan 1963; de Oliveira 1966; Yanoff 1972) and in experimental (Leuvenberger et al 1971, 1974; Cohen et al 1972; Studer et al 1975; Papachristodoulou et al 1976) and spontaneous animal diabetes (Gepts & Toussaint 1967; Duhault et al 1973, 1974). It has been suggested that the loss of these cells is of significance for the development of microaneurysms, but so far there is no evidence for this (Cogan & Kuwabara 1967; Yanoff 1969). The increased ratio between endothelial and mural

cells reflects the decrease in the number of pericytes generally seen in diabetic retinopathy. In contrast mice with the obese hyperglycemic syndrome (obese hyperglycemia and hyperinsulinemia (Westman 1968) displayed no morphological changes in the retinal capillaries (Nieser & Ågren 1978). The differences in pericyte distribution between streptozotocin diabetic mice and obese hyperglycemic mice raises the question as to what extent the insulin values in the latter are of significance for pericyte degeneration.

The enzyme activities in the retinal capillary bed from the streptozotocin diabetic animals found in the present work are similar to those in the obese hyperglycemic mice (Nieser & Ågren 1978). This further emphasizes the previous suggestion that the enzymatic changes may result from the hyperglycemic state. In both human and animal diabetes the differences in enzyme activities may indicate early metabolic changes in the capillaries which are of significance for the later development of diabetic retinopathy.

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## TIMOLOL

*in maintenance treatment of ocular hypertension and glaucoma*

BY

NIELS VESTI NIELSEN and JENS SINDBERG ERIKSEN

The hypotensive effect of Timolol eye drops (0.25 and 0.50%) in maintenance treatment of 64 patients with elevated intraocular pressure has been studied. The patients were treated for a period of mean 13 months. In 44 patients mostly comprising ocular hypertensives a significant reduction in IOP (32%) could be maintained with Timolol alone ( $P < 0.001$ ).

In 20 patients with a high starting baseline pressure or previous maladjustable glaucoma it was necessary to start combined treatment. Pilocarpine, epinephrine or acetazolamide appeared to have additive pressure lowering effect to Timolol.

A significant correlation was present between pre-treatment and Timolol treated intraocular pressures. Thus a pre-treatment IOP above 25 mmHg may indicate a critically hypotensive effect below an IOP of 20 mmHg with Timolol alone.

No significant interference with visual acuity, pupillary size, blood pressure or pulse rate was noted. Existing visual field defects in three patients were slightly aggravated and in four patients with a pathological optic disc visual field loss developed. In seven patients transient sensations of dry eyes and rose-bengal staining dots on the cornea developed.

*Key words:* Timolol maleate – hypotensive effect – combined treatment – one year result – ocular hypertension – glaucoma – side effects

Previous reports on ocular applied timolol maleate (a  $\beta_1$  and  $\beta_2$  blocking agent) have convincingly demonstrated a potent hypotensive effect on elevated intraocular pressure (Bischoff 1978, Katz et al 1976, Kerty & Horven 1978, Krieglstein 1979, Nielsen 1978, Radius et al 1978, Ritch et al 1978, Zimmerman & Kaufman 1977a, b). At the present these results have been based on short term studies.

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Compared to other  $\beta$ -adrenergic blocking agents and other antiglaucoma drugs (pilocarpine epinephrine acetazolamide) Timolol has produced an equal or greater reduction in elevated IOP (Bonomi & Steindler 1973 Kriegelstein 1978 Leidecker 1977 Philips et al 1978 Wettrill 1977). Moreover Timolol has been tolerated well in short term use.

The objective of this study has been to investigate the pressure lowering effect and tolerance of Timolol in maintenance treatment of ocular hypertension and glaucoma. Further the additive effect of Timolol was studied when combined with pilocarpine epinephrine and/or acetazolamide.

### Material and Method

The material consisted of 64 out patients (37 females and 27 males) of various ages (mean 56 years range 30 to 83 years). A baseline IOP  $> 22$  mmHg without topically or systemically antiglaucoma treatment for one week was required before entering this study. In 31 patients we classified ocular hypertension and in 33 patients glaucoma (optic disc cupping and/or visual field loss). Fifty nine patients had open angles three narrow angle glaucoma and two spontaneously partially cured buphthalmos. Aphakic glaucoma was present in three patients. Forty-eight patients had previously been treated with antiglaucoma drugs with a mean duration of 7.8 years (range 0.5 to 29 years). In the remaining 16 patients recent ocular hypertension or glaucoma were detected. Antiglaucoma surgery had previously been performed in 13 patients. The main criteria of admitting the previously treated patients to this study were either a maladjustable glaucoma or discomfort of previous treatment. Any patients with a history of intraocular inflammations or retinal detachment were excluded from this investigation. Further patients with bradycardia ( $< 55$  beats per min) severe heart failure myxoedema bronchial asthma concurrent systemic  $\alpha$ -adrenergic blocking treatment were omitted from treatment with Timolol. Four patients had a mild maturity onset diabetes mellitus.

The study was not designed as a blind trial due to long term treatment of the patients. However the investigator had no knowledge of previous measurements at repeated controls or the concentration and dose of Timolol used in the patients.

In each patient the treatment was primarily started with Timolol 5% administered once daily at 9 p.m. If the pressure lowering effect of this medication was inadequate (i.e.  $> 21$  mmHg) the patients were treated with Timolol 0.5% once or twice daily at 9 a.m. and 9 p.m. In patients where the IOP could not be controlled on Timolol alone we added pilocarpine 1% two or three times daily epinephrine once or twice daily or acetazolamide 500 mg daily.

Measurements of the IOP with Goldmann's applanation tonometry were performed twice at the same time of the day on each control. During a dose adjustment period of three weeks the IOP was controlled at least once weekly. Subsequent controls of IOP during maintenance therapy were performed on the 5th 9th 14th week after the onset of treatment and thereafter with an interval of 12 weeks. In addition to pressure measurements ocular

examinations included visual acuity, visual field, pupillary diameter, slitlamp examination including break up time (BUT) of the tear film and rose bengal vital staining, visual sensitivity, Schirmer test and ophthalmoscopy. Blood pressure and pulse rate were regularly controlled and subjective adverse symptoms if present were recorded.

# Statistical analyses

The hypotensive effect of Timolol was estimated by means of Wilcoxon's signed rank test (two sample tests).

A correlation between pre-treatment baseline pressures and Timolol treated pressures was evaluated by family regression.

## Results

### Intraocular pressure

In 44 patients the IOP was controlled with Timolol alone and in 70 patients combined treatment was instituted. Regarding the duration of therapy, five subjects had been treated for 6-9 months, seven subjects for 9-12 months, 37 subjects for 12-15 months and 15 subjects for 15-18 months.

In the group of 44 patients treated with Timolol alone, a significant reduction in the IOP ( $P < 0.01$ ) could be maintained (Fig. 1). The IOP was reduced with mean 32% after one year of treatment. This group (mean age 63 years, range 43-80

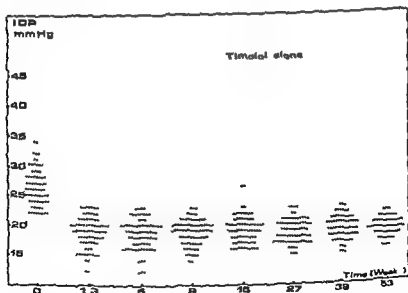


Fig. 1

The scattergram illustrates the hypotensive effect of Timolol alone in 44 patients.

Time (wk)	0	1–3	4	9	18	27	33	53
LOI	mean	31.0	20.8	19.8*	19.1	18.7	21.5**	18.2
	SD	6.0	2.2	2.3	1.8	1.4	6.5	2.1
	n eyes	6	—	—	—	—	—	—
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F	mean	26.5	21.3*	23.3	20.5	21.0	20.5*	18.5
	SD	2.1	3.0	3.1	3.1	1.8	1.7	1.2
	n eyes	4	—	—	—	—	—	—
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E + I	mean	39.5	20.5*	20.7	21.7	20.1	19.0	20.3
	SD	7.7	3.5	3.5	3.4	1.3	1.8	1.7
	n eyes	4	—	—	—	—	—	—
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A	mean	34.3	23.1**	21.1*	20.6	20.3*	19.3	19.7
	SD	9.5	3.8	3.7	3.1	2.9	4.3	3.2
	n eyes	11	—	—	—	—	—	10
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A + I	mean	31.7	22.3	21.7	22.4	21.7	20.3	19.2
	SD	9.4	3.0	3.1	3.1	3.3	3.1	2.1
	n eyes	—	—	—	—	—	—	—
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A + F	mean	31.1	21.1	21.1	21.1	20.0	20.0	20.0
	SD	—	—	—	—	—	—	—
	n eyes	—	—	—	—	—	—	—

indicates the onset of amblyopia.

years) consisted of 16 patients with glaucoma and 28 ocular hypertensives. Thus 90% (28/31) of the ocular hypertensives contrary to 48% (16/33) of the patients with glaucoma were controlled on Timolol alone. No diminution of the initial pressure lowering effect of Timolol on maintenance therapy was noted in these 4 patients ( $P > 0.10$ ).

In 20 subjects it was necessary to combine Timolol with pilocarpine, epinephrine or acetazolamide (Table I). After 1 to 3 weeks of treatment with Timolol 16 patients entered the combined therapy, one patient after 15 weeks and 3 patients after 30 weeks. This group characteristically comprised patients with glaucoma (85%), previously maladjustable glaucoma, high baseline pressures (mean 34.6 mmHg, range 23–60 mmHg) and patients of a high age (mean 71.8 years, range 58–84 years). The mean reduction in IOP with Timolol alone was 11.7 mmHg (39.4% and after onset of combined maintenance therapy 14.6 mmHg (41.9%) (Table I).

In Fig. 2a predictive estimate of the hypotensive response of Timolol compared with baseline pressure before treatment has been attempted. It appears that a starting baseline pressure above 25 mmHg may indicate a critical pressure lowering effect of Timolol alone, when a Timolol treated IOP below 20 mmHg is desirable.

Antiglaucoma surgery had to be performed in two patients after 10 and 24 weeks on treatment with Timolol. In a patient with unmanageable narrow angle glaucoma trabeculectomy was performed, and cycloablation was performed in a patient with an aphakic glaucoma. The latter continued treatment with Timolol.

### Visual field

Glaucomatous visual field loss was present in 24 patients before treatment with Timolol was instituted. After one year on treatment with Timolol a further four patients with an abnormal optic developed a visual field lesion and a slight aggravation of existing visual field defects in three patients.

### Visual acuity

The pre-treatment distance vision was mean 0.80 (range 1.0 to 0.1). No significant changes in visual acuity ( $P > 0.10$ ) were disclosed after 12 months of treatment (mean 0.76, range 1.0–0.01). In nine patients with cataract a slight reduction of visual acuity was noted. One patient was operated for cataract. In general patients previously treated with miotics obtained a better distance vision on Timolol treatment, whereas near vision was disturbed with their former correction.

### Side effects and adverse reactions

Timolol did not induce changes on pupillary diameter or heart rate. No changes in blood pressure or pulse rate was recorded. Investigation of

inter-



BUT and rose bengal vital staining have been reported in a previous paper (Nielsen & Eriksen 1979)

In 10 patients a mild smarting sensation occurred in relation to installation of Timolol. Four patients complained of headache for 2 to 3 h following installation. Seven patients developed transitory dry eyes and innocuous functional and morphological changes (Nielsen & Eriksen 1979). In none of these patients was treatment with Timolol discontinued. After six months of treatment an exudative maculopathy of the left eye occurred in a 70 years male patient with excessive myopia. Visual acuity was severely deteriorated, a large central scotoma was disclosed.

#### Discontinuance from the study

Four patients were withdrawn from Timolol treatment. A 70 years old male patient died from heart failure and pneumonia after 9 months. In two patients surgery, trabeculectomy (24 weeks) and cataract extraction (36 weeks) was performed and further hypotensive treatment was superfluous. The fourth patient did not want to be controlled regularly on Timolol after eight months of treatment.

### Discussion

The present study demonstrates a powerful and prolonged ocular hypotensive effect of topically used Timolol. Thus Timolol seems to be useful in maintenance treatment of elevated IOP. Kriegelstein (1978) and Leydecker (1977) have proclaimed that the hypotensive response of Timolol declines after a short time of treatment (tachyphylaxis) just as is noted with atenolol (Brenkman 1978, Wetrell 1977). We did not disclose any significant diminution of pressure lowering effect in the present material after mean 13 months of treatment. However, in three cases we noted a subsidence of the hypotensive effect or an aggravation of glaucoma after 9-9 weeks of treatment. It should be emphasized that the occurrence of tachyphylaxis may be difficult to assess in clinical studies.

After a dose adjustment period we were able to distinguish two groups of patients with regard to pressure lowering responses of Timolol. In general Timolol proved to have the best effect in the group with a majority of ocular hypertensives, whereas combined therapy chiefly had to be instituted in cases with previously hard manageable glaucomas and high intraocular pressures. In this study as in previous investigations (Bischoff 1978, Kerty & Horven 1978, Nielsen 1978, Radius et al 1978) pilocarpine, epinephrine and acetazolamide proved to have an additive effect to Timolol, also when used in maintenance treatment.

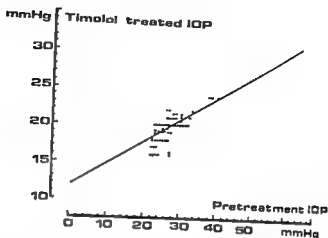


Fig 2

Linear correlation between pre treatment IOP and Timolol treated IOP ( $y = Bx + A$ ,  $10.7 B = 0.32$ ) in 64 patients (121 eyes) on Timolol alone  $P < 0.01$

Taking the total material into consideration a significant correlation was observed between the pre treatment IOP and the IOP obtained by Timolol (Fig 2). Thus from this relationship it may be possible to predict the level of a Timolol treated IOP from a known pre treatment IOP and to estimate whether combined treatment should be expected. Brenkman (1978) has noted an inadequate hypotensive effect of atenolol when pre treatment IOP exceeded 25 mmHg. The pressure lowering response of Timolol alone in the present study may be dubious above pre treatment IOP of 28 mmHg.

Generally Timolol was tolerated well. The dry eyes manifestations were transitory and did not require discontinuance of Timolol therapy (Nielsen & Enkema 1979). Timolol did not significantly interfere with visual acuity. In some of our cases a worsening of existing cataract occurred after one year with Timolol treatment. This may obviously appear without hypotensive treatment. However Timolol seemingly reduces the aqueous formation (Chiou & Zimmerman 1973; Tablonska et al 1978; Zimmerman et al 1978). Prolonged Timolol induced reduction of aqueous secretion and possible changes of aqueous composition may hypothetically interfere with the metabolism of the lens. Treatment with acetazolamide has not induced cataract (Reich 1953). Smith et al (1979) have described a reduction of resting and exercise heart rate during topical treatment with pindolol. We have not, as other investigators (Bischoff (1978), Kerty & Horven 1978, Kriegelstein 1978, Radius et al 1978, Ritch et al 1978, Zimmerman & Kaufman 1977a, b) noted any significant influence of Timolol on blood pressure and pulse rate.

At the present Timolol seems to be a useful drug in the future treatment of elevated intraocular pressure.

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## TREATMENT OF ACUTE OCCLUSION OF THE RETINAL ARTERIES

BY

NIELS VESTI NIELSEN

In five patients with obstruction of the retinal arteries three with emboli the circulation was reestablished by bulbus massage intravenous administered acetazolamide and theophyllamine. The patients were further treated with salicylic acid for six months. An improvement of vision was obtained and maintained over a 2 year period. The basis of the present treatment of occlusive arterial disorders in the retina is discussed.

**Key words:** retinal artery occlusion – ocular massage – hypotensive treatment – theophyllamine – salicylates

The occurrence of retinal arterial occlusion is presumably not uncommon in elderly people. In the study of Karijainen (1971) the incidence of retinal arterial occlusion among outpatients at hospital was about 1% over a period of 11 years. However, sufficient information of the real incidence of arterial obstruction in the retina is not available. Arterial hypertension and arteriosclerosis is frequently present in patients with occlusive vasculopathy in the retina (Lorentzen 1969, Karijainen 1971). Degenerative vasoobliteration and emboli of various origin are the major causes of retinal arterial obstruction (Ffytche 1974).

The treatment of arterial occlusion in the retina has previously been characterised with more or less enthusiasm. Thus various methods (surgical and medical) and in general disappointing results have been reported (Brown & Shields 1979, Ffytche 1974, Kuchle 1977, Magargal & Goldberg 1977, Wise et al 1971).

In this paper an apparently effective management of acute arterial obstruction in the retina will be presented.

## Material and Methods

In five consecutive cases with obstruction of a branch or central retinal artery an immediate treatment was started in the hospital. Temporal arteritis was excluded. The patients were treated regardless of the duration of symptoms of arterial occlusion.

In all patients X-ray of thorax, ECG and stethoscopical examination of the chest and the major arterial trunks of the neck were performed. Haemoglobin, thrombocytes, leucocytes, ESR, serum protein electrophoreses, SM 12 autoanalyser programme and serum lipid, cholesterol, triglyceride were controlled. Ocular examination comprised visual acuity, visual field, slitlamp examination, ophthalmoscopy and photography of the fundus.

### Procedure of treatment

1) *Massage of the bulbous* The eyeball was initially pressed firmly for 15 sec against the bony nasal wall of the orbit while the gaze of the patient was directed downwards. The eyeball was then pressed and depressed abruptly and rhythmically between two fingers with such a force that a deformation of the bulbous could be felt. This procedure was performed for 3 to 5 min in three series unless retinal flow had already been reestablished.

2) *Ocular hypotensive treatment* All patients were treated with acetazolamide 500 mg intravenously on admission, followed by 500 mg perorally daily for 3 months. In 4 patients Timolol 0.1% eye drops twice daily was further used in the acute phase.

3) *Isosartine medical treatment* An intravenous theophyllamine drop (1 g in 500 ml glucose) was administered for 24 h after the massage of the eyeball. On admission a bolus of 200 mg theophyllamine intravenously was administered before treatment with the intravenous drop.

4) *Platelet inhibition treatment* Salicylic acid tablets 1 gram daily for six months were used.

## Case Reports

### Case 1

October 1986 a 74-year-old man was hospitalised for reduction of the left eye vision occurring six h before admission. Since 1972 he had been blind in the right eye owing to a non-treated retinal artery occlusion. Visual acuity was decreased to 6/20 in the affected eye. With the right eye he could perceive hand movements at one meter. In the lower nasal and temporal quadrant of the central visual field the patient recorded a large scotoma in the left eye. On ophthalmoscopy an embolus was localised in the upper macular



Fig. 1a and 1b

Fluorescein angiography illustrating the result of treatment in Case 2. Fig. 1a demonstrates embolic obstruction of the retinal arteries. In Fig. 1b the retinal circulation has been restored after treatment.

arteriole with boxcar phenomena distal from the embolus. The retina was pale and oedematous in the upper part of the posterior pole. Intraocular pressure was 14 mmHg in both eyes and no pathological changes were noted in the anterior segment of the eye. The embolus was dislodged by bulbous massage in 15 min. Next day visual acuity was measured < 6/6 and diminution of scotoma was registered. No somatic pathological findings were present. On follow-up examination two years later the ocular status was unchanged.

### Case 2

A 72-year-old man was admitted 27.07.77 because of blurred vision for four h in the right eye. In two days the patient had several episodes with amaurosis fugax without other neurological symptoms. In 1964 the patient had a severe cranial fracture with optic atrophy of the left eye. Visual acuity was 6/60 in the right and only slight light perception was present in the left eye. Three large paracentral scotomas were registered in the right visual field. Intraocular pressure was 20 mmHg in both eyes. On ophthalmoscopy several arterial emboli were seen in the posterior pole of the right eye. Optic atrophy of the left eye was noted. Fluorescein angiography of the right eye demonstrated obstruction of the retinal arteries in the posterior pole (Fig. 1a). Half an hour after treatment fluorescein angiography showed an improvement of the retinal circulation (Fig. 1b). Next day the visual acuity was improved to 6/6 with 1.0 sph and the scotomas had disappeared. Physical and laboratory findings were normal. At follow-up control in March 1979 no recurrence had appeared.

### Case 3

In a 69-year-old woman a sudden reduction in visual acuity of the right eye occurred 15.09.77 without concurrent ocular or somatic symptoms. The duration of this symptom had been three h. Visual acuity was 6/20 in the right and 6/6 in the left eye with +1.25 sph on both eyes. On the right eye the patient had developed a paracentral scotoma. Slitlamp examination



Fig. 2a and b

The fundus of the patient in Case 3. Complete occlusion of the retinal arteries and diffuse retinal ischemia are present (Fig. 2a). After treatment the retinal flow is improved (Fig. 2b).

and pupillary reactions were normal. Intraocular pressure was 14 mmHg in the right and 16 mmHg in the left eye. In the lower temporal artery of the affected eye an embolus was localized. A systolic cardiac murmur was disclosed. The embolus could be removed by bulbous massage but it reappeared four times during the next two days. On the third day it disappeared. The visual acuity was improved on removal of the embolus to 6/6 with +1.75 sph and the paracentral scotoma remitted. Reexamination in December 1978 showed unchanged ocular findings.

#### Case 4

A 49-year-old woman was primarily admitted 02/08/77 for a total retinal artery obstruction and blindness of the left eye which had been present for two days. At that time no effect of treatment occurred. Two months later the vision of her right eye deteriorated. The time interval before treatment then was three months. Vision of the right eye was reduced to light perception in the temporal visual field compared to 6/6 measured 02/08/77. The pupils reacted slightly on light stimulation. Intraocular pressure was 20 mmHg bilateral. Ophthalmoscopy and fluorescein angiography disclosed nearly complete obstruction of retinal circulation. The retinal circulation was improved after bulbous massage for 30 min. After two days a visual acuity of 3/60 was measured and 14 days later it was 6/94. The patient suffered from an arteriosclerotic cardiac disease with mitral insufficiency. On a reexamination in July 1978 visual acuity was 6/18 and no visual field lesions could be demonstrated.

#### Case 5

A 77-year-old man was admitted 18/01/79 for total blindness of the right eye. This symptom occurred suddenly 19 h before admission. The patient had no other ocular or somatic complaints. One year ago the patient experienced an episode with amaurosis fugax in the right eye. On admission there was no light perception or reaction of the pupil in the right eye. Visual acuity of the left eye was 6/6 with -7.0 sph. The anterior segment of the eye was

normal on slitlamp examination. Intraocular tension was 15 mmHg in the right and 10 mmHg in the left eye. Ophthalmoscopy of the right eye disclosed a pale optic disc and complete occlusion of the central artery with diffuse ischemia of the retina (Fig. 1a). The left fundus appeared normal. After 10 min of bulbus massage the retinal circulation was restored (Fig. 2b). After one week and the following month visual acuity was improved to 20/40 and 20/30 sph. In visual field a central scotoma and a slight peripheral constriction were present. Physical and laboratory examinations disclosed no abnormalities.

## Discussion

The results of the present material indicate that active treatment of arterial occlusion in retina may have a favourable effect. The use of ocular massage apparently had the most convincing and instantaneous effect on occluded retinal arteries. In this series the best visual results 1–2 years after treatment were obtained in 3 cases with emboli localised anterior to the lamina cribosa. In none of the patients was a chorioretinal artery present. It is generally assumed that cloudy swelling of the retinal neurones develops within 3 h after complete arterial obstruction (Ffytche 1974). In this material the majority of occlusions must have been incomplete explaining the effect of treatment despite the rather long duration of impaired vision.

Physical vasodilatation by massage of the globe, a simple method, was already introduced in 1882 by Wood-White. Using this method it is possible to dislodge an atheromatous clot or embolus. Further, a considerable improvement of the arteriolar volume flow (80–180%) has been demonstrated in experimental animals (Ffytche et al. 1974). In the above mentioned cases this method had been performed intensively and repeatedly in each subject as emergency therapy. Thus a rather instantaneous improvement of the retinal circulation was demonstrated with subsequent improvement of vision. Vasodilatation induced by paracentesis of the anterior chamber may presumably have the same effect as bulbus massage (Mingargal & Goldberg 1977; Brown & Shields 1979). However, in animal studies paracentesis has failed to produce more than 20% increase in the retinal volume flow (Ffytche et al. 1974). Moreover, this method is practically more difficult in handling acute arterial occlusion with repeated treatment and continuous registration of the retinal vascular flow.

The use of theophyllamine intravenously as an additive treatment in this material is theoretically supported by the fact that this drug especially provokes a vasodilation in normal cerebral vessels thereby increasing the flow to the focal diseased areas – inverse intracerebral steal phenomenon (Skindin & Paulson 1970). The vasomotoric tonus of the retinal vessel in many respects is autoregulated by the same mechanism as the cerebral vessels (Wise et al. 1971).



The administration of vasodilating drugs apparently may have an autoward effect on the cerebral and possibly the retinal circulation due to a marked vasodilatation of the normal vessels and subsequent decreased flow within the obstructed area — steal phenomenon (Skinhøj & Paulson 1970 Wise et al 1971). However a vasodilatation may be useful when vasospasms predominate especially in young people.

Salicylic acid was used with the aim of reducing the risk of a thromboembolic recurrence by inhibition of platelet aggregation (Harrison et al 1971). During the past years evidence has accumulated that antiplatelet treatment may be beneficial in cerebrovascular thromboembolic disease (Didisheim et al 1974).

Only few reports have dealt with the spontaneous course of untreated arterial occlusion in the retina (Kuchle 1977 Magargal & Goldberg 1977). It must be stressed that controlled prospective blind studies are required to assess the significance of active treatment surgical and pharmacological compared to the results of untreated arterial obstruction in the retina.

The results of the present series indicate however that ocular massage and intravenous administered acetazolamide a simple treatment could reasonably be performed in patients with retinal arterial occlusion before admission to hospital.

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## OCULAR LESIONS IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA

BY

INGE VASE<sup>1</sup> and POUL VASE<sup>2</sup>

A regional survey has been carried out in order to establish the prevalence of hereditary haemorrhagic telangiectasia (HHT) (Rendu-Osler-Weber's disease). Forty-seven patients fulfilled the proband criteria. No patient was registered due to ocular symptoms.

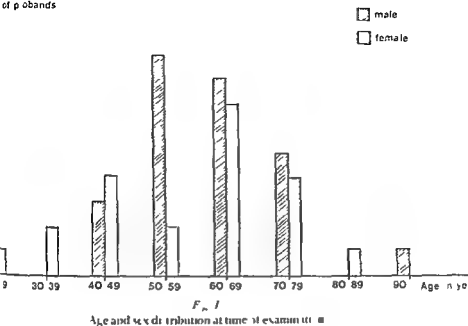
All of the probands were subjected to ophthalmological examination in order to estimate the distribution of ocular lesions in HHT. Conjunctival lesions were found in 20 cases, and an intraocular vascular abnormality in only one case. The possibility of intraocular lesions being a component of the clinical picture of HHT or a sporadic vascular abnormality is discussed.

**Key words:** hereditary haemorrhagic telangiectasia - Osler's disease - conjunctival lesions - retinal lesions.

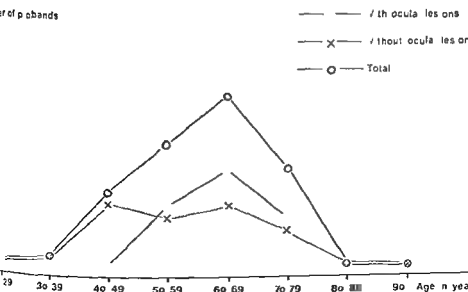
Hereditary haemorrhagic telangiectasia (HHT) is a rare autosomal dominant hereditary disease with typical vascular abnormalities in respect of histopathology and localization. The telangiectases are localized to the venules and capillaries in the skin and mucosa. Recurring epistaxis is a predominant trait, but bleeding may occur from the conjunctival sac, oral mucosa or gastrointestinal tract.

Several reports have appeared, mainly regarding conjunctival telangiectases since the first investigations on ocular lesions in HHT (Chaffard 1896, Weber 1907). The first description of intraocular vascular lesions was given by Gjessing (1911) and later by François (1938). There have been few reports on retinal lesions and these are mainly divided into two groups: partly varicose venules and paravascular haemorrhages (Cuendet & Magnenat 1953, Landau et al. 1956, Calmettes et al. 1958) and partly arteriovenous aneurisms (Forster & Bean 1963, Davis & Smith

of p bands



of p bands





*Fig 3*

Typical conjunctival lesions in Osler's disease with clusters of telangiectases

venule was observed during ophthalmoscopy. Some tortuosity but no haemorrhage was seen in this area. The vascular deformity in the right eye was demonstrated by means of fluorescein angiography.

Multiple lesions were found in the conjunctivae in another case with severe affection of the skin and mucous membranes. Ophthalmoscopy of this patient showed no signs of intraocular vascular deformities and although fluorescein angiography was carried out, no abnormality was observed.

### Discussion

There is good general agreement in respect of the conjunctival lesions between the findings of the present investigation and reports in the literature as to the pronounced similarity between the conjunctival lesions and the mucosal lesions elsewhere in the system.

The intraocular lesions, however, show a more heterogeneous picture. Gysin (1915) described retinal paravascular haemorrhages in a 26-year-old man suffering from severe vitamin C deficiency secondary to recurrent epistaxis. In 1949 Francois described

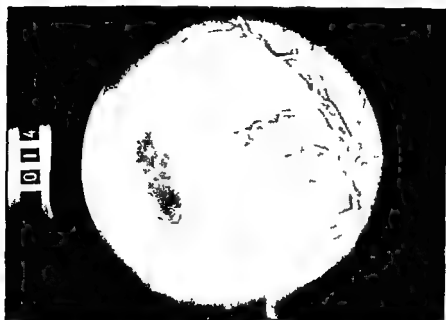


Fig. 4

Fluorescein angiogram showing an arterio-venous shunt between retinal artery and upper temporal venule in the right eye of a 67-year-old woman with Osler's disease

retinal vascular lesions with dilated and tortuous retinal venules in a 74-year-old woman with hypertension. Landau et al. (1956) found bilateral varix-like venous ectases in the retina of two sisters. In 1963 Forker & Bean found a unilateral retinal vascular abnormality in a 19-year-old man with tortuosity of the venules simulating a complex arterio-venous aneurysm.

Ehlers & Jensen (1973) suggested a mesodermal dysplasia as the common cause of different retinal lesions. However, the intraocular vascular lesions differ considerably in occurrence and variability from the remaining clinical manifestations of HHT.

The present authors, however, are of the opinion that the retinal lesions are a rare vascular abnormality in a normal population rather than a part of the clinical picture of hereditary haemorrhagic telangiectasia.

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## CONGENITAL HORIZONTAL GAZE PARALYSIS FACIAL HEMIATROPHY AND SITUS INVERSUS OF THE OPTIC DISC

### A case report

BY

LEONARD ROTHKOFF<sup>1</sup> and BENZION BIEDNER<sup>2</sup>

A case of congenital horizontal gaze paralysis in association with facial hemiatrophy and situs inversus of the optic discs is described. Except for the presence of deafness on the side of the atrophic face no other neurologic abnormality was present. To our knowledge no similar case has been reported.

**Keywords:** gaze paralysis — facial hemiatrophy — situs inversus

Congenital horizontal gaze paralysis has been described in association with facial palsy in some cases of Mobius Syndrome (Hicks 1943) and in a case of Klippel Feil syndrome (Witzel 1958). The purpose of this report is to describe a congenital gaze disturbance with facial hemiatrophy and situs inversus of both optic discs.

### Case report

A six year-old boy was referred to us with the diagnosis of congenital esotropia. He was born after a normal pregnancy and delivery to unrelated parents. There is no family history of ocular abnormalities. Psychomotor development has been normal.

On examination asymmetry of the face was apparent, with the left side moderately atrophic as compared to the right side (Fig. 1). This was confirmed by X rays which showed hypoplasia of the mandible and zygoma on the left side (Fig. 2). The left ear was smaller than the right, with a preauricular nodule and atresia of the external meatus (Fig. 3). Audiometry revealed moderate hearing loss on the left side due to a conduction defect.

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Fig 1

Facial asymmetry caused by hemiatrophy of the left side. Note smaller ear on this side. Eyes appear to be straight in the primary position.



Fig 2

Facial X-ray showing hypoplasia of the mandible and zygoma on the left side.



Fig 3

Preauricular nodule and malformed left ear.



Fig 4

Full vertical movement. Top looking up, bottom looking down.





Fig 5

Attempted lateral gaze. Top looking in the right, bottom looking to the left.  
Note retraction of the non fixing eye

Since birth on the posterior scalp near the midline a cauliflower like soft nodule measuring  $2 \times 2 \times 2$  cm had been present which was originally diagnosed as a meningocele but on re-evaluation at the age of three years was felt to be a dermoid with an intracranial sinus tract. On plain skull films no intracranial lip dermoid cyst was seen.

Visual acuity was 6/6 in both eyes. On straight ahead gaze the eyes were orthophoric (Fig 1). Vertical movements were normal (Fig 4). However no lateral movement could be elicited. Instead attempts at horizontal gaze were replaced by convergence movements (Fig 5) in association with pupillary constriction and moderate retraction of the non fixating eye. Optokinetic response could be obtained vertically but not horizontally. Similarly caloric stimulation could not elicit any horizontal ocular movement. Convergence occurred normally with no retraction noted upon near point testing. Forced ductions were normal.

Ophthalmoscopy revealed situs inversus of both discs (Fig 6). The macula and periphery were normal.

Neurologic examination was normal with no evidence of facial nerve palsy found. The rest of the physical examination showed no abnormalities.

### Discussion

The congenital gaze disturbance described in this case is similar to that previously described (Zweifach et al 1969) and is characterized by 1) absence of version on attempted conjugate lateral gaze 2) the use of convergence instead of adduction for lateral viewing 3) retraction of the non fixing convergent eye on attempted lateral



Fig. 6

Situs inversus of optic discs Top right eye Bottom left eye

gaze 4) and the presence of normal vertical movements. As in our case these findings may be misinterpreted as a congenital esotropia with cross fixation if not for the presence of orthophoria in the primary position and the presence of the near reflex with ocular retraction on attempted lateral gaze.

The retraction of the convergent non fixing eye on attempted lateral gaze is similar to the prominent sign in Duane's Syndrome. Paradoxical innervation of both horizontal recti has been shown in the retracting eye in such cases (Breuninger 1957) and was alluded to in a case such as ours where electromyography was attempted (Zweifach et al 1969). It is noteworthy that narrowing of the palpebral fissures was not present in our case.

The presence of vertical movements and convergence in the absence of horizontal gaze even with optokinetic and caloric testing, can be explained by the

separate anatomic centers for these functions. Our case would be typical of a lesion in the pons affecting the horizontal gaze centers while sparing the midbrain centers for vertical movement and convergence.

Failure of lateral movements of both eyes is usually associated with bilateral facial paralysis in the congenital Möbius Syndrome. Despite the presence of facial hemiatrophy, no evidence of facial nerve involvement was present. The anomalies of the external ear and conduction deafness are typical in this condition (Duke Elder 1964). The etiology of facial hemiatrophy is unknown. It has not previously been reported in association with horizontal gaze palsies.

Situs inversus of the optic disc is a congenital anomaly whereby the central retinal vessels emerge toward the nasal instead of the temporal side. Though often associated with additional ocular defects such as myopia, inferior conus (Caccanuse 1954), temporal field defects, refraction anomalies and amblyopia (Manor 1974), we could find no report of motor anomalies except for that of exotropia (Fishman et al 1976).

A common pathogenesis for these three anomalies not previously associated is not apparent. It is generally accepted that horizontal gaze palsies are due to a lesion in the pontine centers for lateral gaze (Zweifach et al 1969). Various hypotheses have been presented for the facial hemiatrophy, including a lesion of the seventh nerve, but none has been proven (Duke Elder 1964). The situs inversus, a form of diversion of the optic nerve, is seemingly unrelated to the other anomalies and is not present in other family members. The most that can be proposed is a common embryologic factor acting early in fetal life.

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## CHORIORETINITIS OF THE NEWBORN WITH HERPES SIMPLEX VIRUS TYPE 1

Report of a case

BY

PER REERSTED and BENTE HANSEN

A disseminated herpes simplex virus (HSV) infection involving the central nervous system and accompanied by chorioretinitis in a 3 week-old girl is described. The aetiological diagnosis was established on the basis of virus isolated from skin vesicles and a significant rise in complement fixing antibodies to HSV type 1.

The mode of transmission of the virus to the infant apparently was direct contact with an oral lesion in the mother that was present at the time of delivery. The patient survived but became blind and microcephalic with severe neurological sequelae.

The virus isolated was identified as HSV type 1 which is an infrequent finding in herpetic chorioretinitis of the newborn.

*Key words:* chorioretinitis—herpes simplex virus—type 1—newborn

Since Cogan et al (1964) published a study of retinopathy attributable to HSV in a 3 week-old infant this subject has attracted considerable attention. According to Cibis (1975) 14 out of 160 reported cases of neonatal HSV infection had chorioretinitis. However none of the typed cases with chorioretinitis were associated with HSV type 1 infection (Nahmias et al 1977).

The present report describes a case of disseminated infection with CNS involvement accompanied by chorioretinitis in a 3 week old girl. The virus cultured was identified as HSV type 1.

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Received April 30 1979

## Case Report

A 3-week-old girl was admitted under the diagnosis of malnutrition to Childrens Hospital Blegbakken Copenhagen on November 9th 1977

The infant was born in the 36th week of gestation weighing 9800 g. Three days before admission she was noted to be quiet and unresponsive. Vesicles were observed on her left cheek and on her chest.

A clinically manifest maternal HSV infection was reported at the time of delivery. A large vesicle on the upper lip persisted for 2-3 weeks of nursing. No evidence of prior herpetic infections was recorded. Two months after delivery the mother developed dendritic keratitis in her left eye. She was treated at the Department of Ophthalmology, Municipal Hospital of Copenhagen with topical application of iododeoxyuride and adenine arabinoside after which the lesion vanished.

On admission the infant was lethargic with low body temperature (34°C). Vesicles in clusters were seen in the upper right part of her chest and a few lesions were found on her left cheek. There was no involvement of the oral cavity or the eyes. On the second day she developed seizures and on the following day the course was stormy with fever, seizures, difficulties of breathing and vasomotor instability. Her breathing had to be assisted mechanically. There were signs of disseminated infection with hepatomegaly and pathological liver parameters. A few more vesicles appeared until the eighth day.

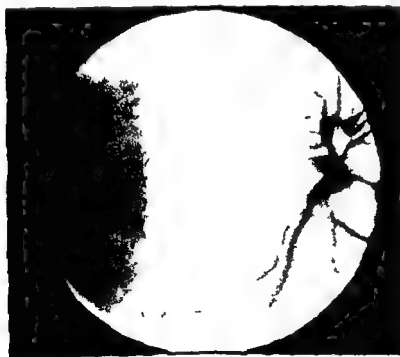


Fig. 1  
Central scarring (right eye)

Five and six months later small vesicles recurred on the left cheek. They were treated with iododeoxyuridine ointment. Systemic treatment with antiviral preparations was omitted.

The patient is still alive but she is blind and microcephalic with severe neurological sequelae.

*Ophthalmological examinations* Ophthalmoscopy on the fourth day and six weeks after admission was normal. A third examination under general anaesthesia at the age of 14 weeks however showed bilateral chorioretinitis in the equatorial and central regions. The vitreous was clear and the optic disc seemed without atrophy. Centrally the foveola was atrophic with a halo of pigmentation (Fig. 1). In the equatorial region circumscribed chorioretinal scarring most prominent in the upper parts was seen (Fig. 2). The scars were composed of severely hypertrophic pigmentation and white atrophic areas. The protrusion was approximately three dioptres. The lesions were somewhat more widely distributed in the left eye.

*Virological studies* Viral cultures from the initial skin vesicles revealed HSV. They were typed by the mouse hepatitis test (Mogensen et al. 1974) as HSV type 1.



Fig. 2  
Part of equatorial scarring (right eye)

were cultures from skin lesions appearing seven months later. Complement fixing antibody titers to HSV type 1 rose from "negative" on the second day of admission to 1:240 seven weeks later. The maternal antibody titer to HSV 3½ months after delivery was 1:60. Scrapings from the cervix uteri six weeks later showed no signs of virus.

*Additional investigations.* A lumbar puncture on the day of admission showed no cells or bacteria. No bacteria or viruses were cultured. IgC was within normal limits. IgM and IgA were subnormal. EEG was diffusely abnormal. Toxoplasma titers of mother and child were negative.

## Discussion

Herpes simplex virus exists in two closely related antigenic types. HSV type 1 is primarily associated with nongenital infection, whereas HSV type 2 is associated with genital infection together with occasional infection of the newborn (Nahmias & Roizman 1973).

HSV infection of the newborn is a serious condition with a high fatality rate, and severe sequelae are common in survivors. Among the sequelae reported, those involving the CNS and the eye predominate (Nahmias et al. 1973).

Since most cases of neonatal HSV infection are acquired on passage through an infected birth-canal, the majority of isolated virus strains (>70%) belong to the genital type of the virus (HSV type 2), whereas the oral type (HSV type 1) accounts for only about 30%. The source of the virus in the latter cases may either be genital HSV type 1, maternal infection, a non-genital maternal infection or a non-maternal one (Nahmias et al. 1973).

The present history of recognizable maternal HSV lesions on the lips at the time of delivery and in the left cornea a few weeks after delivery is highly suggestive of a maternal infection caused by HSV type 1, as was actually isolated in the infant. The onset of symptoms in the reported case falls within the range of the incubation period of neonatal HSV infections from birth to three weeks reported by Nahmias et al. (1973). This and the rise in HSV antibody titer from negative on the second day of admission to 1:240 50 days later is strongly indicative of postnatal transmission of the virus from a presumably oral infection in the mother. An infection in the mother's genital tract cannot, however, be excluded.

The retinal lesions observed 2½ months after the initial infection are presumably congenital, indicating a previous acute chorioretinal infection (Cibis et al. 1978).

Nahmias et al. (1973) stressed that the number of reported neonatal HSV type 1 infections was now large enough to permit a preliminary view of whether or not the clinical spectrum varies according to HSV type. They did not find any striking

differences with the two virus types in 89 cases of HSV type 2 and 40 cases of HSV type 1 infection with the one exception of chorioretinitis having so far been observed only with HSV type 2.

The present case seems to be among the first ones to show chorioretinitis in a neonatal HSV type 1 infection indicative of common clinical appearance of the two HSV types in chorioretinitis of the newborn.

### Addendum

The patient died of her sequelae April 1979 and unfortunately autopsy could not be done.

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## IN VITRO PRODUCTION OF STEROID CATARACT IN BOVINE LENS

### Part I Measurement of optical changes

BY

DAVID MILLER, M. L. TJERINA and CHAIM MAYMAN

Fresh calf lenses were incubated in nutritive media to which was added ouabain and dexamethasone phosphate at concentrations of  $1 \times 10^{-4} M$ . At the end of a three day incubation period cortical opacification developed in the control series of lenses as well as those lenses incubated in both ouabain and dexamethasone phosphate. Using a light transmission device which quantitates lens opacification it was noted that dexamethasone produced a level of cortical opacification significantly greater than that of the control series. Ouabain produced a level of cortical opacification statistically identical to that produced by the dexamethasone. It is suggested that the aforementioned light transmission device is an accurate and reproducible method of quantitating cataractous opacification.

**Key words:** steroid cataract - in vitro cataract - cataract quantification - ouabain cataract - cataract turbidity

Research on cataracts has been conducted by scientists representing medicine, biochemistry and physics. Collecting and comparing the results of research from these disciplines is important if cross fertilization of ideas is to take place. To this end we feel that standardizing the measurement of the severity of the cataractous process is an important first step in encouraging communication between researchers.

In this paper we intend to (a) describe a simple and precise method for measuring experimental cataracts and (b) use the technique in assessing the severity of cataracts produced by ouabain (Bonting et al 1963; Bonting 1965; Palva &

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measured. Preliminary studies showed that light incident to the lens was scattered by all portions of the lens. Thus the light received by the photomultiplier probe gathered a representative sample from all portions of the experimental lenses.

## Results

Table 1 presents the light transmission data in arbitrary units for each group of differently treated lenses along with a comment on the statistical difference between each series and the control.

Table 1 reveals that both dexamethasone and ouabain induce opacities of the same degree.

Fig. 2 demonstrates the appearance of typical lenses in the control (C), ouabain (OUA), dexamethasone (DEX) and fresh (F) groups.

Both the ouabain and dexamethasone induced opacities were significantly greater than the control lens opacity.

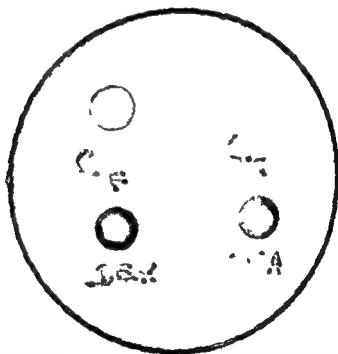


Fig. 2

The appearance of calf lenses after 3-day incubation. Top left: fresh control lens. Top right: incubated control lens (3 days). Lower left: lens incubated in  $1 \times 10^{-4}$  M dexamethasone. Lower right: lens incubated in  $1 \times 10^{-4}$  M ouabain. Whiteness signifies transparency and blackness signifies opacity.

In each case the opacity was always located in the lens cortex leaving the lens nucleus clear. Using the dissecting microscope set at 30 $\times$  magnification one could see that the individual lens fibers of the cortex were opaque as was the layer of epithelial cells just under the lens capsule. If the fresh lens were left in incubation media plus dexamethasone for two hours small vacuoles were noted to form along the boundary between cortex and nucleus.

Thus from a macroscopic point of view the experiment seems to demonstrate the following series of events in a lens incubated in culture medium. First there is an accumulation of vacuoles which ultimately coalesce at the border between the cortex and nucleus. Then an opacification of the cells and fibers of the cortex develops. Finally a peeling off of the opacified cortex takes place leaving a clear nucleus.

### Discussion

The present study follows the cortical opacification of fresh calf lenses incubated for three days in nutritive media. The process appears to be accelerated by a factor of 7 if either ouabain or dexamethasone at a concentration of  $1 \times 10^{-4}$  M is added to the medium.

Since the degree of lens opacification produced by dexamethasone was identical to that produced by ouabain in our series of experiments and since ouabain has been shown to inhibit  $\text{Na}^+ \text{K}^+$  ATPase in the lens the data suggest that both may act via the same mechanism that of inhibition of  $\text{Na}^+ \text{K}^+$  ATPase in the lens.

We also noted that use of the light transmission apparatus described in the study gave precision to the measurement of cataract opacification far beyond the 1 to 4+ system conventionally used.

### Acknowledgments

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## IN VITRO PRODUCTION OF STEROID CATARACT IN BOVINE LENS

### Part II Measurement of sodium potassium adenosine triphosphatase activity

BY

CHAIM I. MAYMAN, DAVID MILLER and M. L. TJERINA

Fresh calf lenses incubated in nutrient media containing dexamethasone phosphate or ouabain in concentrations ranging from  $1 \times 10^{-4}$  M to  $1 \times 10^{-8}$  M developed cortical opacification and showed significant inhibition of  $\text{Na}^+$   $\text{K}^+$  ATPase activity. Over a 3-day incubation period the decrease in  $\text{Na}^+$   $\text{K}^+$  ATPase activity correlated well with the observed decrease in light transmission. The degree of enzyme inhibition and decrease in light transmission varied directly with the concentration of dexamethasone phosphate and ouabain, with significant changes observed at physiologic and pharmacologic concentrations of these agents. Lenses incubated for 4 days in dexamethasone phosphate or ouabain showed substantial increases in water content as well as an increase in  $\text{Na}^+$  and a decrease in  $\text{K}^+$  concentration. These data suggest that inhibition of the cation pump may play a significant role in the formation of steroid cataract *in vitro*.

**Key words:** dexamethasone – inhibition – light transmission –  $\text{Na}^+$   $\text{K}^+$  ATPase – ouabain

An extensive clinical experience has shown that cataract formation occurs in some patients receiving long term steroid therapy for conditions such as rheumatoid arthritis, asthma, and nephritis (Gullberg & Elman 1973; Loredi et al 1973; Kennedy 1970; Benati & Ball 1971; Kristensen 1968). It has been found to occur rather frequently in children, even those on short term and low-dose therapy (Behari & Grossman 1968; Taub 1968; Braver et al 1966). It has also been shown

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that topical corticosteroid administration in the therapy of ophthalmic disease produces a similar effect. The cataract produced under these circumstances is typically posterior subcapsular in location and presents a serious problem to those afflicted in this manner. However, the mechanism of steroid cataract formation remains obscure. In recent years Kinsey, Kinoshita and others (Kinsey 1973; Kinoshita 1974; Iwata & Kinoshita 1971) in studying various types of cataract formation have stressed the importance of cation fluxes across the lens and their relation to the cation pump of which the enzyme Sodium Potassium Adenosine Triphosphatase ( $\text{Na}^+ \text{K}^+ \text{ATPase}$ ) is an important component. Maintenance of the pump/leak balance is considered crucial to the preservation of the viability and transparency of the lens. Interference with pump function by the specific inhibitor ouabain (Bonting 1965; Bonting et al. 1963) or in the Nakano strain of mice (Iwata & Kinoshita 1971) causes accumulation of water and the formation of a cataract. Some authors have shown (Harris & Becker 1965) that steroids exert an effect on cation balance in the lens. The mechanism, however, of the change in cation balance has not been elucidated.

Recently Mayman and Tijerina have reported an inhibitory effect of the steroid dexamethasone 21 phosphate on the  $\text{Na}^+ \text{K}^+ \text{ATPase}$  of choroid plexus (Mayman 1972, 1974; Mayman & Tijerina 1979). This inhibition is of the same order of magnitude as that of ouabain. Postulating that inhibition of this enzyme in the lens and thereby of the pump should produce cataract formation, Müller et al. (1979) reported in the previous paper cataract formation when calf lenses were incubated for three days in medium containing  $1 \times 10^{-4}$  M dexamethasone 21 phosphate. The cataract thus formed was of the same degree and type as that produced by incubation in equimolar concentrations of ouabain.

The present communication reports significant inhibition of  $\text{Na}^+ \text{K}^+ \text{ATPase}$  in cataractous lenses incubated in media containing as little as  $1 \times 10^{-4}$  M dexamethasone 21 phosphate. The inhibition produced by dexamethasone 21 phosphate is similar to that produced by ouabain. In the body of this paper the term dexamethasone refers to the use of dexamethasone 21 phosphate.

## Material and Methods

Calf eyes obtained from a local abattoir were delivered to the laboratory within one to two hours after the death of the animal. The lenses were removed atraumatically from the enucleated globe and homogenized in a glass homogenizer in 0.05 M Tris buffer containing 0.05 M EDTA. The homogenate was centrifuged at 13000 g for one hour according to the method of Kinoshita (1974).  $\text{Na}^+ \text{K}^+ \text{ATPase}$  activity of the insoluble fraction was measured according to the methods of Samson & Quinn (1967) and Lowry & Lopez (1949) in the presence of varying concentrations of dexamethasone and ouabain.

In another series of experiments fresh calf lenses were prepared and incubated in

dually in medium containing varying concentrations of dexamethasone in a manner described in the foregoing communication (Miller et al 1979). Lenses were also incubated in media containing similar concentrations of ouabain. Control incubations of lenses were carried out in identical media containing neither dexamethasone nor ouabain.

At intervals during a three-day incubation period the lenses were removed from the medium and light transmission measured in the manner previously described (Miller et al 1979). After measurement of light transmission whole lenses were homogenized in 0.05 M tris buffer pH 7.4 containing 0.05 M EDTA using a teflon coated homogenizer. The insoluble fraction was collected by centrifugation of the homogenate at 13000 g for 60 min in a Sorvall RC2 II centrifuge. The residue was resuspended in 1.0 ml of 0.05 M tris buffer pH 7.4 and used for  $\text{Na}^+$   $\text{K}^+$  ATPase assay.

The  $\text{Na}^+$   $\text{K}^+$  ATPase assay based on the measurement of inorganic phosphate (Pi) liberated by the  $\text{Na}^+$   $\text{K}^+$  ATPase activity is carried out in the following manner. The complete incubation medium of 1.0 ml contained tris buffer 0.05 M pH 7.4, ATP 5.0 mM,  $\text{MgCl}_2$  5.0 mM,  $\text{NaCl}$  100 mM and  $\text{KCl}$  9.0 mM. The reagent medium was equilibrated at 37°C for 5 min and the reaction begun by addition of 25  $\mu$ l of tissue homogenate. Incubations were carried out in a water bath at 37°C for 5 min and the reaction stopped by placing the reaction mixture in ice. Aliquots were removed and Pi measured according to the method of Lowry & Lopez (1946). The  $\text{Na}^+$   $\text{K}^+$  stimulated ATPase activity was determined by the difference in activity in the presence and absence of  $\text{Na}^+$  and  $\text{K}^+$ . Formation of inorganic phosphate (Pi) was found to be linear with time over this incubation period. Appropriate standards and blanks were treated in an identical manner. A zero time tissue blank was run by placing tissue in the reagent medium and stopping the reaction immediately.

The protein content of the tissue suspensions was determined by the method of Lowry et al (1951).

To assess the water content of lenses after incubation in dexamethasone or ouabain ( $1 \times 10^{-4}$  M) for four days lenses were removed from the incubation medium, patted dry with filter paper, weighed, then heated at 120°C until steady weight had been obtained.

In a similar series of experiments  $\text{Na}^+$  and  $\text{K}^+$  content was measured in lenses incubated for three days in dexamethasone or ouabain. After incubation lenses previously exposed to each type of medium were pooled and homogenized in a glass homogenizer in ten volumes of water. The homogenate was centrifuged and filtered. An aliquot of the filtrate was used for  $\text{Na}^+$  and  $\text{K}^+$  measurements in a flame photometer.

## Results

Fig. 1 shows the  $\text{Na}^+$   $\text{K}^+$  ATPase activity of the 13000 g insoluble fraction in the presence of varying concentrations of dexamethasone and ouabain. A pharmacologic dose response curve is seen with concentrations ranging from  $1 \times 10^{-4}$  to  $1 \times 10^{-8}$  M. It can be seen that dexamethasone inhibits most of the ouabain-inhibitable portion of the enzyme. At concentrations that are considered to be pharmacologic (i.e.  $1 \times 10^{-5}$  M) 60 per cent inhibition occurs with lower levels of inhibition occurring at lower concentrations.

When lenses were incubated for three days in media containing either dexamethasone or ouabain ( $1 \times 10^{-4}$  M)  $\text{Na}^+$   $\text{K}^+$  ATPase activity was measured at daily

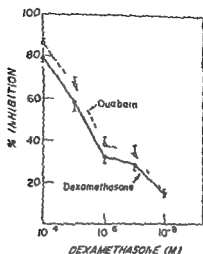


Fig. 1

The inhibitory effect of dexamethasone and ouabain on  $\text{Na}^+\text{K}^+$  ATPase of calf lens. Vertical bars indicate standard error of the mean. The numbers of lenses used in these experiments were as follows:

$10^{-8}$ M	20	$10^{-7}$ M	10
$10^{-6}$ M	10	$10^{-5}$ M	10
$10^{-4}$ M	10		

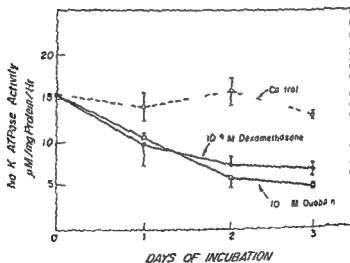


Fig. 2

Changes in  $\text{Na}^+\text{K}^+$  ATPase activity in lenses incubated in dexamethasone or ouabain-containing media for 3 days. Vertical bars indicate standard error of the mean. The numbers of lenses used in these experiments were as follows: Lens 0 time = 0

Day 1 = C (6)	Dex (1)	Oua (4)
Day 2 = C (6)	Dex (4)	Oua (1)
Day 3 = C (6)	Dex (1)	Oua (1)



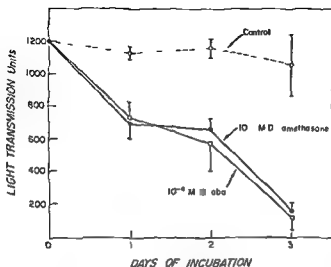


Fig 3

Changes in light transmission through lenses incubated in dexamethasone or ouabain containing media for 3 days. Vertical bars indicate standard error of the mean. The numbers of lenses used in these experiments were as follows: lenses 20.

Day 1 = C (6)	Dex (4)	Oua (4)
Day 2 = C (6)	Dex (4)	Oua (4)
Day 3 = C (6)	Dex (4)	Oua (4)

intervals over this period. These data were correlated with daily light transmission measurements as described in the previous communication (Vuller et al 1979). Figs 2-3 illustrate the changes observed over this period. It is clear that control lenses show only slight decrease in light transmission over the three-day incubation period while lenses incubated in dexamethasone or ouabain show a marked decrease in light transmission. These changes in light transmission correlate well with a decrease in Na<sup>+</sup> K<sup>+</sup> ATPase activity observed over the same period of time. There was an initial change in both light transmission and enzyme activity. By the second day, enzyme activity was 47 and 37 per cent of control in the case of lenses incubated in dexamethasone and ouabain respectively. Light transmission was reduced to 57 per cent in ouabain containing media. Thus the changes in light transmission and enzyme activity were of similar magnitude over this period of time.

At the end of the three-day incubation, enzyme activity was 51 per cent of control with dexamethasone and 38 per cent in the case of ouabain. Therefore, the greatest percentage decrease in enzyme activity occurred in the first 48 h of incubation while the greatest relative decrease in light transmission occurred between 48 and 72 h of incubation.

Table I

Light transmission in calf lenses incubated in dexamethasone or ouabain (percentage)

Concentration	Control	Dexamethasone	Ouabain
$1 \times 10^{-3}$ M	100	$17 \pm 2$ (21)	$3 \pm 1$ (11)
$1 \times 10^{-4}$ M	100	$14 \pm 1$ (54)	$15 \pm 1$ (34)
$1 \times 10^{-5}$ M	100	$11 \pm 1$ (21)	$9 \pm 2$ (17)
$1 \times 10^{-6}$ M	100	$91 \pm 2$ (18)	$91 \pm 3$ (18)
$1 \times 10^{-7}$ M	100	$67 \pm 11$ (10)	$49 \pm 1$ (10)

Numbers in brackets represent number of lenses in each experiment

Tables I and II show the effect on light transmission and ATPase activity in lenses incubated for three days in dexamethasone or ouabain at concentrations ranging from  $1 \times 10^{-3}$  to  $1 \times 10^{-7}$  M. The figures are expressed as percentages of the data for the control lenses.

The results of measurements of water content in lenses incubated in dexamethasone or ouabain containing media are shown in Table III. Fresh lenses had a water content of 69 per cent — a figure consistent with the findings of previous investigators (Harris & Gruber 1962). Lenses incubated in control media had approximately the same water content (69%) while those incubated in dexamethasone or ouabain containing media ( $1 \times 10^{-4}$  M) showed sharp increases of 12 and 73 per cent respectively.

Ion content of lenses incubated in varying concentrations of dexamethasone or ouabain are shown in Table IV. Fresh controls show a  $\text{Na}^+$  concentration of 14 and  $\text{K}^+$  concentration of 130 mEq/l. Three day control lenses show some increase in  $\text{Na}^+$  and  $\text{K}^+$  loss. However lenses incubated in dexamethasone show a great increase in  $\text{Na}^+$  and a decrease in  $\text{K}^+$ . Lenses incubated in varying concentrations of ouabain show changes in ion content similar to those observed with dexamethasone.

Table II

 $\text{Na}^+$   $\text{K}^+$  ATPase activity in lenses incubated in dexamethasone or ouabain (% effect)

Concentration	Control	Dexamethasone	Ouabain
$1 \times 10^{-3}$ M	100	$10 \pm 1$ (21)	$18 \pm 5$ (11)
$1 \times 10^{-4}$ M	100	$24 \pm 2$ (38)	$26 \pm 9$ (3)
$1 \times 10^{-5}$ M	100	$33 \pm 2$ (21)	$23 \pm 3$ (11)
$1 \times 10^{-6}$ M	100	$46 \pm 3$ (18)	$57 \pm 3$ (18)
$1 \times 10^{-7}$ M	100	$74 \pm 5$ (13)	$90 \pm 7$ (13)

Numbers in brackets represent number of lenses in each experiment

*Table III*

Water content of lenses incubated for 4 days (% of total weight)

Control (fresh)	69 ± 0.7	(20)
Control (incubated)	69 ± 2	(8)
Dexamethasone (1 × 10 <sup>-4</sup> M)	73 ± 1*	(8)
Quabain (1 × 10 <sup>-4</sup> M)	75 ± 1	(8)

Numbers in brackets represent number of lenses in each experiment

 \*  $P < 0.01$     \*\*  $P < 0.025$ 
*Table IV*

Ion content after 3-day incubation (mEq/L)

Control (fresh) (17)			Na <sup>+</sup> 18 ± 0.2		K <sup>+</sup> 130 ± 0.7	
	10 <sup>-4</sup>		10 <sup>-5</sup>		10 <sup>-6</sup>	
	Na <sup>+</sup>	K <sup>+</sup>	Na	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>
Control (incubated)	24 ± 5 (10)	122 ± 2 (11)	24 ± 5 (11)	190 ± 2 (11)	25 ± 1 (9)	123 ± 0.5 (9)
Dexamethasone	84 ± 3 (26)	66 ± 10 (26)	62 ± 11 (12)	87 ± 15 (12)	65 ± 4 (9)	80 ± 1 (9)
Quabain	83 ± 12 (26)	60 ± 9 (26)	68 ± 29 (12)	83 ± 28 (12)	62 ± 19 (9)	89 ± 19 (9)

## DISCUSSION

A number of authors including Kinoshita (1974) and Kinsey (1965) have commented on the importance of the cation pump mechanism in maintaining the viability and normal transparency of the lens. Since the Na<sup>+</sup> K<sup>+</sup> ATPase is thought to be an important component of the pump, interference with this enzyme would lead to a breakdown of the normal pump-leak balance. In the case of the specific inhibitor of Na<sup>+</sup> K<sup>+</sup> ATPase, ouabain, and in the Nakano strain of mice, inhibition of the enzyme has been found to be associated with cataract formation.

The data presented in this communication show that calf lenses incubated for

three days in media containing ouabain or dexamethasone undergo cataractous changes which are accompanied by a marked decrease in  $\text{Na}^+/\text{K}^+$  ATPase. Serial measurements of enzyme activity show a gradual decrease which correlates well with the decrease in light transmission noted over the same period of incubation. It is also shown that with both dexamethasone and ouabain there is a pharmacologic effect since a concentration of  $1 \times 10^{-5}$  M causes greater inhibition of enzyme activity than  $1 \times 10^{-7}$  M.

Previous work by Harris & Becker (1965) has shown a loss of  $\text{K}^+$  and an increase in  $\text{Na}^+$  in rabbit lenses incubated in prednisolone phosphate  $1 \times 10^{-5}$  M at 0 and 37°C. More recently Friedburg et al. (1974) have shown *in vivo* an increase in  $\text{Na}^+$  concentration in the lens when methyl prednisolone was injected into the vitreous of guinea pigs. These changes would be consistent with a reduction in the activity of the cation pump and would suggest an inhibition of the  $\text{Na}^+/\text{K}^+$  ATPase of the lens. It is of interest therefore that our experiments showing an inhibition of the enzyme also demonstrate an increase in  $\text{Na}^+$  and a loss of  $\text{K}^+$  ion. The present work appears to confirm our original postulate namely that dexamethasone might exert an inhibiting effect on the cation pump.

The concentrations of dexamethasone used in these experiments are in the range of  $1 \times 10^{-5}$  M to  $1 \times 10^{-7}$  M. It may be argued that concentrations of  $1 \times 10^{-5}$  M or  $1 \times 10^{-4}$  M are a magnitude or two higher than would be expected at physiologic or pharmacologic levels. However, since similar effects may be seen in our *in vivo* experiments at concentrations of  $1 \times 10^{-5}$  M– $1 \times 10^{-7}$  M it would suggest that these observations may have physiologic significance. Moreover in an experimental study designed to assess the amounts of steroid delivered to the eye by various vehicles Green & Downs (1974) found that the concentrations of prednisolone phosphate administered to the cornea of rabbits was of a similar order of magnitude ( $1\text{--}4 \times 10^{-5}$  M) used in some of our studies.

Other effects of steroids on the lens are of course also possible. It is known that dexamethasone interferes with glucose uptake into lymphosarcoma P1798 cells but hexokinase is not inhibited (Rosen et al. 1970). The mechanisms involved in this process are at present obscure and a membrane effect is thought to be involved. In addition steroids are known to induce certain enzymes such as tyrosine amino transferase (Gerschenson et al. 1970; Cranner et al. 1970) and phenylethanolamine N-methyl transferase (PNMT) (Pohorecky et al. 1970) in certain tissues such as liver, fat cells and adrenal gland. The effect of steroids such as aldosterone on RNA metabolism and protein induction (RNA polymerase) are also well known (Fraser 1971). It is not known at this point whether these effects are related to the work reported here.

Clinical experience has shown that steroids such as dexamethasone 21 phosphate have an important cataractogenic effect. The mechanisms involved have been

obscure. In the preceding communication it was shown that a steroid induced cataract could be experimentally produced. It was postulated that inhibition of  $\text{Na}^+ \text{K}^+$  ATPase might be an important mechanism involved in this process. The present work shows that a significant inhibition does in fact occur under these experimental conditions. Observations on ion concentrations and water content further suggest that inhibition of the cation pump may play a significant role in the formation of steroid cataract *in vitro*.

### Acknowledgments

The authors would like to thank Dr. Leo T. Chylack for his helpful advice in the conduct of this study. We would also like to express our gratitude to Professor George Benedek and Dr. Jin Kinoshita for their comments and suggestions during the course of this work.

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**Documenta ophthalmologica Proceedings Series Volume 18 Progress in Anterior Eye Segment Research and Practice** Dr W Junk b Publishers The Hague Boston London 1979 Edited by O Hockwin & W B Rathbun 370 pages Price US dollar 75 —

Volume 18 in the Documenta Ophthalmologica Proceedings Series is in honour of Prof John E Harris University of Minnesota and consists of submissions from invited authors working with anterior eye segment research and practice. The volume is an excellent compilation of current concepts in this field of ophthalmology and includes the latest results from a great number of outstanding workers. It is to be recommended.

*Preben Gilbert*

**Hulf Ehrlich: Atlas der Kontaktlinsen** 1979 and Enke Verlag Stuttgart 1979  
169 pages 443 illustrations 19 —

Contact lens fitting is a practical subject. Photographs can often tell more than pages. This atlas is a picture book. The author's conversational form. Case histories and faceted subject are omitted. The book is a systematic exposition. Few aspects of this subject are omitted. The physical, chemical and optical properties of the basic materials and disinfection and product control are covered. The book is a practical guide to contact lens fitting and design examinations necessary prior to fitting techniques for soft lenses and rigid lenses. Special consideration is given to bandage lenses and iris lenses.

Further information is given in the appendix. The book is recommended to everybody interested in contact lens practice.

*A. Dreyer*

**Darold J Apple and Maurine F Rabb: Clinical-pathologic correlation of ocular disease: a text and stereoscopic atlas** 2nd ed 36 pages 83 illustrations and 112 stereoscopic views in colour on 16 View Master reels Mosby St Louis 1978 Price US dollar 158.75

This new edition has been expanded with approximately 400 new clinical photographs and photomicrographs and there are 30 new colour stereophotographs. The atlas correlates excellently the clinical and pathological findings in ocular diseases and the many illustrations are most instructive.

The book is to be warmly recommended to all student ophthalmologists. Every ophthalmic department ought to have an example in its library.

A View Master is available in any toyshop — Dkr 32 —

*S R Andersson*

*Bayan Ranka Vitamin A und Auge Abhandlungen aus dem Gebiete der Augenheilkunde Sammlung von Monographien Band 17 Georg Thieme Leipzig 1979 31 pages, 13 illustrations and 4 tables Price DM 19.80*

Vitamin A a vital food constituent for man and for animals was identified at the beginning of this century. In spite of extensive research and a large scale production of this vitamin, blindness is still due to lack of vitamin A. Figures of 100 000 new blind children every year because of A avitaminosis are mentioned and in some countries e.g. India, the number of affected persons is huge. A presentation and discussion of the ophthalmologic problems related to lack of vitamin A and to overdoses of vitamin A is therefore still absolutely relevant.

The author gives a survey of vitamin A occurrences in food and in organism and its metabolism. The main section of the book is a presentation of the ophthalmological symptoms of vitamin A deficiency. This is very useful. The chapter on vitamin A in ophthalmological practice hardly stands to a scientific criticism e.g. it is claimed that retinitis pigmentosa may be effectively treated by vitamin A (1). The final chapter on hypervitaminosis A is quite short. Chronic as well as acute intoxication is discussed, but the possible occurrence of papilloedema is not mentioned.

The book would seem to be of interest to those particularly engaged in the treatment of avitaminosis A or in research on eye complications in vitamin A deficiency.

*Vol. 11*

*J. M. Emery Current concepts on cataract surgery. Selected proceedings of the fifth biennial cataract surgical congress C. V. Mosby Comp. St. Louis 1978*

These proceedings include 197 brief and concise articles on almost every aspect of cataract surgery. All the major headlines are well-known: intracapsular extraction, extracapsular extraction, intraocular lenses, operative complications, postoperative complications, correction for aphakia and results of cataract surgery.

It may be worthwhile to mention a few of the topics. The discussions on outpatient surgery are interesting. The choice of surgical procedure may be determined by several factors, but in the northern European countries there would seem to be severe economical arguments in favour of outpatient cataract surgery. Other topics discussed are phacolytic glaucoma and intraocular lens implants. Techniques are still under development and the long term effect of the corneal endothelium after these procedures still await elucidation.

There can be little doubt that for a practically working ophthalmologist it would be extremely useful to participate in such a congress and the participant may afterwards in the proceedings look up any particular matter that he might want to recall. However the writer cannot keep back the impression that the report will be of a limited benefit for the unprepared readers as well as for the writers and that the real interest in the publication of these congress proceedings is on the part of the publishing companies. Regular reading of a few of the more important ophthalmic journals would seem to give the same amount of information.

With this criticism the present volume may still be found useful due to its discussion which take up a substantial part of the pages.

*Vol. 11*



*Rudolf Sachse* *eneger* Kompendium und Atlas der Augenheilkunde Gustav Fischer Verlag Stuttgart, New York 179 pages 278 figures

The author's intentions – to facilitate intensify and rationalize the teaching of medical students in the growing field of ophthalmology – have been completed by limiting the content to a level which corresponds to the knowledge required from Danish medical students. Furthermore the text is short though sufficient and comprehensible. Though the text is compressed and the letters are small a good layout enables the students to see the book quickly. Recapitulations and small tables have the same purposes.

This book should be considered in the training program for medical students.

*To the Editor*

*Georg Eisner* Ophthalmologische Operationsindikationen Eine Orientierung für die gemeinpraktiker Verlag Hans Huber Bern Stuttgart Wien 1979 81 pages 80 illustrations Price Fr 68 – DM 76 –

This is a manual for the general practitioner who wishes to obtain a summary insight into the principles of modern ophthalmic surgery as far as indications, procedures and consequences are concerned. To the general practitioner the section on photocoagulation in diabetic retinopathy may be of particular interest. Recent advances such as vitrectomy, corneal transplantation, Cuppers Faden operation in strabismus and incomitant squint are probably dealt with in too much detail for the general practitioner. On the other hand such every day ophthalmological problems as cataract are mentioned too briefly (e.g. nothing about corneal incisions or indications for or difficulties in intraocular lens implantation, too little about the problems about combined cataract and glaucoma operations). Nothing is stated about the surgical treatment of concomitant squint.

Thus in many respects the book is a valuable guide for the general practitioner in countries where it is difficult for the patient to contact an ophthalmologist. Maybe the book might also be of interest to practising ophthalmologists who have not been in touch with ophthalmological surgical practice for the past 10–15 years or more.

*Anders Bruun Laurson*

*Arthur Siu Ming Lim and Ian J. C. Little* Colour Atlas of Ophthalmology H. K. Lippincott London 1979 151 pages 193 illustrations (189 in colour) Price Eng pds 6.00

A small handy book with handsome well-chosen instructive colour photographs on matt paper. Most eye diseases are represented several patients are exotic (one of the authors comes from Singapore). An amusing picture shows a Chinese superstition: Counter irritant placed on the forehead above an eye with acute glaucoma.

The text is concise and like a compendium. It is instructive and up-to-date. The reviewer disagrees with the authors on only one or two points. There are few misprints (Scotometer and Goldman perimeter figures are reversed, pilocarpine (C)).

The book is suitable as additional reading for students, ophthalmological assistants and nurses.

*Mogens Vorn*

## V A R I A

### *The International Society for Clinical Electrophysiology of Vision (ISCEV)*

will hold its XVIIIth Symposium in Amsterdam from 18th to 22nd May 1990

The three main subjects of the symposium are

- a) peripheral processing emphasizing on pathology and physiology
- b) neural pathways e.g. routing recording from subcortical structures
- c) central processing especially contributions on binocularity and pathology will be appreciated

The symposium language will be English. Offers of papers are kindly requested there will be facilities for scientific demonstrations and posters

For further information and application forms please write to  
Professor H. Spekrijse, Netherlands Ophthalmic Research Institute  
P.O. Box 6411, 1007 EA Amsterdam, The Netherlands

### *Consilium Europaeum Strabismi Studio Deditum*

The Xth Meeting of the CESSD will take place at The Institute of Child Health, Guilford Street, London WC1, on April 18th and 19th 1990. Prior to the European Ophthalmological Congress in Brighton.

Enquiries and information may be obtained from Miss Barbara M. Lee, MBE, Moorfields Eye Hospital, High Holborn, London WC1 7AN.

(Cont. from cover page 2)

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